Longitudinal Analysis of the Pubertal Growth, Body Composition, and Endocrine Development in Young People with and without Diabetes

Thesis

Longitudinal Analysis of the Pubertal Growth, Body Composition, and Endocrine Development in Young People with and without Diabetes

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Abstract

This longitudinal study of children with type 1 diabetes (T1D) and contemporary controls through puberty attempts to elucidate differences in their growth, pubertal development and the relationship with pubertal hormones.

Fifty two T1D prepubertal children aged 7.7 - 14.4y and 125 control children aged 8.3 - 11.96y were recruited. Auxology and puberty were assessed and blood samples were obtained 6 monthly until the age of 16 years. Annual bone age was assessed in T1D subjects.

Puberty onset and PHV was later in T1D boys compared to controls, while T1D girls were younger at puberty onset with an advanced bone age and their peak height velocity was earlier compared with controls. Overall compared to the UK references, T1D girls had a reduced peak height velocity whereas the boys had normal pubertal growth.

T1D children had greater increases in BMI during puberty but whereas in the girls this was due to greater acquisition of fat mass, in the boys it related to gains in fat free mass.

Levels of DHEAS, IGF-I, A4, testosterone and oestradiol were lower in T1D boys and girls whereas leptin and SHBG were higher.

The pubertal delay in T1D boys was partially explained by bone age but diabetes presence was also contributory. The earlier puberty, advanced pubertal bone age and reduced height gain in T1D girls were associated with complex hormonal changes. The advanced bone age at pubertal onset was not influenced by adrenal hormones but by BMI. Loss in height gain in these girls was related to E2 levels and glycaemic control at the start of puberty.

This study helps clarify some of the relationships between hormonal changes and variation in auxological factors during normal puberty. Differences in these parameters between T1D and control children are only partially explained by endocrine relationships; other factors, relating to T1D including glycaemic control and insulin dose, are important.

This work is dedicated to David and Mike,
a long wait hopefully justified.

But also to the memory of Clara whose optimism and dreams she passed on to her children.

"Who was your mother?"
"Never had none!" said the child, with another grin.
"Never had any mother? What do you mean?
Where were you born?"
"Never was born!" persisted Topsy
"Do you know who made you?"
"Nobody, as I knows on,"
said the child, with a short laugh
"I 'spect I grow'd"

Harriet Beecher Stowe
Uncle Tom's Cabin 1852

Outline of Contents

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And last but of course not least, many thanks to my family for their continuing support and belief in me in this long endeavour.

List of abbreviations

T1D	Type 1 diabetes mellitus
E2	Oestradiol
GH	Growth hormone
GHBP	Growth hormone binding protein
IGF-I	Insulin-like growth factor-1
IGF-BP1	IGF binding protein-1
IGF-BP3	IGF binding protein-3
A4	Androstenedione
SHBG	Sex hormone binding globulin
DHEAS	Dehydroepiandrosterone sulphate
DHEA	Dehydroepiandrosterone
FAI	Free androgen index
FT4	Free thyroxine
TBG	Thyroid binding globulin
HbA1c	Glycosylated haemoglobin
TEM	Technical error of measurement
SEM	Standard error of the mean
se	Standard error of a particular statistic
SD	Standard deviation
SDS	Standard deviation score
P	Probability (that a result is significant)
PHV	Peak height velocity
LMS	L=power transformation to make Gaussian M=Median curve, S= coefficient of variation
CDGP	Constitutional delay of growth and puberty
B	Regression coefficient
β	Standardized regression coefficient
CA	Chronological age
BMI	Body mass index
FM	Fat mass
FFM	Fat free mass
%BF (or PCBF)	Percent body fat
B	Breast
G	Genitalia
PH	Pubic hair
M	Menarche
GnRH (or LHRH)	Gonadotrophin releasing hormone
DXA	Dual energy x-ray absorptiometry
TBW	Total body water
D_2O	Deuterium oxide
TBK	Total body potassium
MLwiN	Multi-level modelling
CAH	Congenital adrenal hyperplasia
DKA	Diabetic ketoacidosis

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Chapter 1. Introduction

1.1 Normal Human Growth

1.1.1 The Human Growth Curve: Growth in terms of Karlberg's ICP model

The curve of human physical growth results from a complex interaction of cells responding to messages from the brain via neurotransmitters, from endocrine glands via hormones, from adipocytes via leptin and other adipocytokines, from the environment via nutritional intake as well as social/emotional circumstances and from the genetic endowment bestowed on the individual.

The characterisation of the shape of the human growth curve has varied with time and differing analysis. It was undoubtedly the work of Frank Shuttleworth (1899-1958) who elegantly defined the shape of the curve based on his analysis of a large number of children aged 5 to 17 years from the Harvard School of Education Study. His work was based on that of Boas (1858-1942) who earlier had discovered that individuals' curves had a different shape to those of averaged curves. It was Shuttleworth who clearly showed the difference between longitudinal studies and cross-sectional ones with his diagram of height velocity showing individual curves centred around the age of maximum velocity and the mean curve based on average velocities of a number of different children (Tanner 1999). The latter presents a curve that is flattened and spread out along the age axis losing entirely the concept of individuality.

Postnatal growth is characterised by a period of rapid but decelerating growth during the first three years of life. This is followed by a relatively quiescent period of slow deceleration through childhood reaching a nadir before the

tumult of the pubertal growth spurt. This is superbly demonstrated in the first known recorded longitudinal study of growth (1759-1777) on an individual, that of Count de Montbeillard's son (Tanner 1962). Measurements were taken every 6 months from birth to 18 years of age and in addition to the three phases of growth just mentioned also demonstrates the often smoothed over mid-childhood spurt that occurs between 6 and 8 years of age in many children (Butler et al. 1990).

The mathematical analysis of these phases of growth by Karlberg (Karlberg et al. 1987a; Karlberg et al. 1987b) into three distinct functions corresponds to the accepted understanding of the endocrinology of the growth process (Hindmarsh and Brook 1995). According to Karlberg, the first component of the model is that of 'infancy' and is described by an exponential function that is largely nutrition dependent. The second phase of 'childhood' begins from an average age of 9 months and is depicted by a second-degree polynomial regression that is felt to be growth hormone dependent. The third phase of 'puberty' described by a logistic function is hormonally dependent on both growth hormone and the sex steroids (either directly or through their effect on growth hormone). Karlberg did not include the mid-childhood growth spurt (claimed by Molinari et al (Molinari et al. 1980) to occur in 2/3rds of children) in his model since he claimed it couldn't be used to predict puberty. Although disputed, some investigators (Hindmarsh 2002) claim that this can be explained by an increase in adrenal androgens at this time prior to a true pubertal initiation.

1.1.2 The midchildhood growth spurt

The existence of a midchildhood growth spurt, occurring between the ages of 6-8 years, has often occasioned controversy. In a detailed analysis, Butler,

McKie and Ratcliffe (Butler et al. 1990) reviewing the literature found that although a spurt between 6 and 8 years of age was often demonstrated in width and circumference measurements (Molinari et al. 1980; Tanner and Cameron 1980) its recurrent presence for height was disputed (Tanner and Cameron 1980; Meredith et al. 1981; Berkey et al. 1983). The work of Bock and Thiessen in 1980 (cited by Butler et al 1990) on the Berkeley California data and the mathematical analyses of Molinari et al and Gasser et al on the Zurich Longitudinal Growth Study (Stuetzle et al. 1980; Gasser et al. 1985) has, however, indisputably demonstrated its presence. Studying 80 boys and 55 girls from the Edinburgh Longitudinal Growth Study, Butler et al (Butler et al. 1990) observed a midchildhood spurt in all the children except one girl. They also found evidence for two further spurts; one they called the pre-school spurt which occurred in 92.5% of boys and 81.8% of girls at mean ages of 4.8 and 4.6 years respectively, the other they labelled the late-childhood spurt which was observed in 96% of boys and 80% of girls at 9.2 and 8.6 years respectively. Bock (Bock 2004) analysing data on 167 boys and 152 girls from the Fels Longitudinal Study found similar results of multiple prepubertal growth spurts using different analytical methods compared to Butler et al. He commented that the generality of this phenomenon occurring at random times, though more frequent in some children than others, points to the view that they are unrelated to any physiological developmental stage i.e. adrenarche. This agrees with Remer and Manz (Remer and Manz 2001) who claimed that no previous longitudinal study had ever been performed looking at both the hormonal and auxological parameters of the mid-childhood growth spurt. They studied 19 children seen annually with auxology and urine samples (although

the final analysis for this purpose was only done on 8 children) and observed a significant overall increase of adrenal androgen secretion from 2 years before the spurt to 2 years after. However, 'after multiple testing' they concluded that significant increments were only seen 1 year after the spurt (for DHEAS) or 2 years after (for 17 ketosteroids) and therefore could not be the primary cause of the accelerated growth. They did, however, comment that higher androgen levels such as those seen in premature adrenarche could have a growth accelerating effect.

1.1.3 Pubertal Growth and Peak Height Velocity

Pubertal growth, the third phase of Karlberg's model, encompasses the pubertal growth spurt, which will be the main focus of this thesis. This spurt, also termed 'peak height velocity' (PHV) is the fastest annual velocity during puberty calculated over a twelve-month period and centered over the year of occurrence. When the calculation is done on data that has been graphically or mathematically smoothed to reduce measuring error, seasonal variation and overcome the problem of missing data, it is referred to as 'instantaneous peak height velocity'. Measurements taken every 3-4 months during this period will maximise the accuracy of the assessment but due to practical considerations, those taken six monthly are often used. There are a number of widely accepted facts concerning PHV observed by Tanner et al (Tanner et al. 1966a; Tanner et al. 1966b) in British children but also by others in different populations, these include: PHV invariably occurs approximately 2 years earlier in girls than boys and at different stages of puberty between the sexes (early in girls and late in boys); there is a marked sex difference in the magnitude of the spurt (boys greater than girls) and those children who have an earlier spurt

have a more vigorous spurt than those who have a later spurt. Growth velocity reaches its nadir in the year before the spurt and this is usually more evident in boys than girls (Tanner et al. 1966a). Although most boys will grow 5cm/yr in the year preceding their spurt, some may decelerate sharply and grow more slowly at 3.5cm/yr. It is widely held that children who begin puberty early (i.e., age 7yrs in girls and 9yrs in boys), are taller before puberty, shorter at the onset of puberty but have a final height similar to later maturing children (Karlberg et al. 1987b; Vizmanos et al. 2001).

1.1.3.1 Timing

The age at which peak height velocity occurs is a definite landmark in the growth process of each individual but can only be ascertained retrospectively. This age varies from country to country (and from region to region within a country) and depends on the circumstances of the individual. In boys in the south of England this occurs at a mean (sd) age of 13.9 (0.9) years and is relatively late in the pubertal process. In girls, PHV occurs at the beginning of puberty with the mean age of occurrence in England at 11.9 (0.9) years (Tanner et al. 1966b). Although the timing of the onset of puberty (first appearance of B2/G2) is not very different between the sexes (11.15 y in the girls and 11.64y in the boys (Marshall and Tanner 1969; Marshall and Tanner 1970), the two-year difference in the timing of PHV means that there are often several years when many girls will be taller than their age matched male contemporaries. This is a short-lived phenomenon but for many youngsters a difficult rite of passage to endure. Most of the final height difference between the sexes is attributed to this two-year difference (Tanner et al. 1976).

1.1.3.2 Magnitude

There are different ways that PHV is calculated that yields different values. The mean (sd) whole year peak height velocity-centred peak velocity is 9.5 (1.1) cm/yr in boys and 8.4 (0.9) cm/yr in girls (Tanner et al. 1966b). This is velocity calculated over a whole year (and is less than the instantaneous peak height velocity), centred on the peak that includes 6 months before and 6 months after it. Using an automated algorithm, Coste et al (Coste et al. 2002) found not only similar ages for PHV in both boys and girls; 13.9 (0.9)y and 12.2 (0.9) y respectively but also PHV magnitudes very similar; 9.6 (1.3) cm/y and 8.4 (1.3) cm/y respectively. When an attempt at ascertaining the exact moment of instantaneous peak height velocity is made, measurements every 3 or 6 months in themselves are not adequate and a smoothed curve must be fitted either graphically or mathematically. The instantaneous PHV on the Harpenden data was 10.3 (1.5) cm/yr in boys and 9 (1.0) cm/yr in girls (Tanner et al. 1966b).

The magnitude of peak height velocity is greater the earlier it occurs and there is a negative relationship between the magnitude and its age of occurrence in both sexes. Tanner et al (Tanner et al. 1966a) found the regression of PHV on the age it occurs to be -0.77 in boys and -0.47 in girls. This means that for every year of age of later occurrence of PHV, there is a decrease in magnitude (b ± se) of 0.77± 0.21 cm/yr in boys and 0.47± 0.17 cm/yr in girls. There is therefore less difference in the peaks of early and late spurting girls than there is for boys. This negative relationship of magnitude of PHV to its age of occurrence is a universal finding although the exact regression varies.

1.2 Normal Puberty

'Puberty' defined as 'that period of time in which adolescents reach sexual maturity and become capable of reproduction'. The origin of the word is from the late Middle English, from the Latin, *pubertas* related to *pubes* or pubic hair (The New Oxford Dictionary of English 1998).

In their seminal papers on patterns of puberty in girls and boys, Marshall and Tanner (Marshall and Tanner 1969; Marshall and Tanner 1970) meticulously described the normal variation of pubertal events in each sex. These observations were based on longitudinal data on 192 girls and 228 boys in a children's home seen every three months during puberty in their Harpenden Growth Study that ran from 1948-1972. The genitalia (G), breasts (B) and pubic hair (PH) were rated according to 'Tanner' stages (Tanner 1962) with stage 1 being pre-pubertal and stage 5 being adult. These were adapted and simplified from the work of Reynolds and Wines (Reynolds and Wines 1948; Reynolds and Wines 1951) in their study of growth and development from the Fels Longitudinal Study (Yellow Springs, Ohio, 1929 - current). Pubic hair and genitalia / breast were each given a separate rating in recognition that they were under different hormonal control. Marshall and Tanner (Marshall and Tanner 1969; Marshall and Tanner 1970) demonstrated that although there was great variation in individuals in the timing of the onset of puberty and great overlap among the different events, there remained an overall synchrony.

1.2.1 Stages of Puberty

1.2.1.1 Girls

Ages on reaching the different stages of puberty

The ages from Marshall and Tanner (Marshall and Tanner 1969) at first appearance of each pubertal stage is shown in the following table:

	B2	PH2	PHV	B3	PH3	PH4	B4	M	PH5	B5
mean	11.15	11.69	12.14	12.15	12.36	12.95	13.11	13.47	14.41	15.33
sd	1.10	1.21	0.88	1.09	1.1	1.06	1.15	1.02	1.12	1.74

Table 1.2a, Girls: Age at first appearance of each pubertal stage, Marshall and Tanner

These puberty ratings were done from the standardised photographs taken of each child at each visit, the authors commented that the age of PH2 was probably later than in real life due to the difficulty in assessing the first appearance of this stage on a photograph. There was also a comment that although the mean age of menarche in this group was observed to be 13.47 years, it is generally 4 months (0.3y) earlier in girls raised in normal conventional home environments in the south of England. In fact in the earlier 1966 British Standards paper, Tanner et al reported the mean age of menarche as 13.2y but felt a more appropriate age was 13 (quoting data from the London County Council) and so used this as the age of menarche that best represented urban British girls.

Time to go through puberty

The longitudinal nature of the Harpenden study and the frequency of visits allowed the possibility of examining the length of time it took for each girl to pass through the various stages of puberty. Looking at the interval from B2 to B5 it was observed that some girls might take 1.5 years to traverse this and others could take more than 6 years although the average tended to be about 4

years. A girl could begin breast development slowly and continue until stage 4 but the time spent in stage 4 was not related to the length of time spent in the earlier stages. Tanner and Marshall (Marshall and Tanner 1969) made the interesting speculation that the breast might be under different hormonal controls in early puberty compared to later puberty. The average interval between B2 and menarche is approximately 2.5 years but this can range from less than a year to up to nearly 6 years. There appears to be no clear relationship between the age a girl begins to develop and the time she takes to get to full maturity.

Age and the length of time to go through puberty

This question of whether children who mature early move through puberty faster was addressed by Marshall and Tanner by looking at the interval from the beginning of breast development (B2) and age of menarche. One is early in puberty and the other relatively late and so can be used as an indicator of time in puberty. The correlation coefficient between the age midway between the two [(B2 age+Menarcheal age)/2] and the time interval between them (Menarcheal age-B2 age) was 0.17, not significantly different from zero. "Thus the interval between the two events does not appear to be related to the age at which they take place."

In conclusion, it is the sequence of events, although not exactly the same for each individual, that is less variable than the age at which the events occur. It appears that the relationship between the different pubertal events is a more important indicator of normality than the actual age at which they occur.

1.2.1.2 Boys

Ages on reaching the different stages of puberty

Similarly to the girls, puberty in the boys develops sequentially from one stage to the other with no stages being omitted.

	G2	G3	PH2	G4	PH3	PHV	PH4	G5	PH5
mean	11.64	12.85	13.44	13.77	13.9	14.06	14.36	14.92	15.18
sd	1.07	1.04	1.09	1.02	1.04	0.92	1.08	1.10	1.07

Table 1.2b, Boys: Age at first appearance of each pubertal stage, Marshall and Tanner

Again Marshall and Tanner (Marshall and Tanner 1970) commented that the age for the appearance of pubic hair (PH2) was unlikely to be accurate due to the difficulty of noticing this stage on photographs. Of interest to point out is that between the ages of 13 and 14 they noted that normal boys could be found in any stage of sexual development.

Time to go through puberty

There is great variation in the time taken to pass through puberty. The average for passing from G2 to G5 was 3.05 years with a range from 1.86 to 4.72 years. There is also great variation in the time spent in any one stage of puberty, for example G2 in some boys will last 0.4 years and in others 2.2 years.

The extent of the variability with which individual boys pass through puberty is such that one may go from G2 to G5 in less time than another may take to go from G2 to G3. The use of photographs to assess the beginning of pubic hair development inevitably gives rise to errors and data from the Dutch study of Van Wieringen et al (van Wieringen et al. 1968) was used with a mean age of 11.75 years (instead of the 13.44y from the photos) for the appearance of PH2 (using the Harpenden study sd of 1.1y).

Age and the length of time to go through puberty

To examine if the time taken to pass through puberty was related to the age at which puberty occurs the regression of the interval G2-G5 on the age at the midpoint of this interval was calculated and was not significantly different from zero. Therefore, as in the girls there is no relation to the speed with which a boy progresses through puberty and the age at which it occurs.

1.2.2 Growth during puberty

1.2.2.1 Introduction

Rapid changes in size, shape and body composition characterise the pubertal years in both sexes. Increased linear growth rate and sexual differentiation are the hallmarks of this period. Girls reach each stage of puberty before boys although there is a great deal of individual variation. From the preadolescent dip or nadir in the growth velocity (approximately 1 year before PHV) to final height there is a gain (mean, sd) in height of 28 (3.5) cm in boys and 25 (4.1) cm in girls (Marshall and Tanner 1969; Marshall and Tanner 1970). Approximately 16% (11-21%) of adult final height can be attributed to height attained during the growth spurt (Tanner et al. 1976).

1.2.2.2 Sexual dimorphism

The sexual dimorphism in adult height in England is approximately 13cm and it is claimed that most of this ($2/3^{rds}$) is due to the two-year later onset of the male pubertal growth spurt (Tanner et al. 1976). Hauspie et al (Hauspie 2002) analysed the dynamics of sex differences on various measurements in British children. They found a 12 cm difference in adult height of which 7.9 cm was due to the later onset of the spurt in the boys, with 2.1cm attributable to sex differences in prepubertal growth and 2cm to differences in the adolescent gain.

A mathematical analysis using smoothed spline functions by Largo et al (Largo et al. 1978) on 222 children (112 boys) from the Zurich Longitudinal Study (1955-1976) observed that the adult sex difference in height of 12.6 cm in the Swiss population could be attributed to: +1.6cm by more prepubertal growth in boys, +6.4 cm by the boys' delayed spurt, +6.0 cm by the more vigorous spurt in boys and –1.4 cm by more post-spurt growth in girls. An analysis by Gasser et al (Gasser et al. 2000) found that 48% of the adult sex difference in height could be due to the later onset of the pubertal growth spurt in the boys. There appears to be a consensus that it is the later spurt of the boys that is the most significant factor in their greater final height although the exact proportion varies not only between populations but with differing analyses within a population.

1.2.2.3 Final Height

There is some controversy as to how the age of pubertal onset affects final height. Several investigators have reported that the timing of puberty does not affect final height (Largo et al. 1978; Zacharias and Rand 1983; Tanner and Davies 1985; Stanhope et al. 1988; Vizmanos et al. 2001), although Hagg and Taranger (Hagg and Taranger 1991) observed that delayed puberty resulted in taller young men (although no difference in final height in their girls). A large American study of girls by Biro et al (Biro et al. 2001) using menarche as a marker for early, average and late maturers found that those Caucasian girls who had an early menarche (<11.7 years) were shorter as adults than those who had an average (11.7-13.5 years) or late menarche (>13.5 years) by 1.2 and 2.6 cm respectively. They also found that the early maturers gained more height after menarche than later maturers but that this was balanced by the

shorter prepubertal growth period. Consistent with most published research they also observed that early maturers had greater peak height velocities.

1.2.3 Tempo / Relationship with growth

1.2.3.1 "Tempo"

Franz Boas (1892) was the first to introduce the concept of 'tempo of growth' as well as that of 'physiological age' (Tanner 1999). This is the now well known observation that children do not all develop at the same rate; that some are early developers while others are late developers who will for the most part catch up their earlier developing peers. In his 1989 book 'Foetus into Man' (Tanner 1989), JM Tanner said that this analogy came from classical music where some children grow *andante* (moderate tempo), others *allegro* (quick tempo) and still others *lentissimo* (slow tempo). As previously mentioned, the velocity curves of Frank Shuttleworth (Tanner 1962) markedly illustrated the different conclusions that could be drawn when pubertal growth was assessed without allowing for the individuality of each child's growth curve.

1.2.3.2 Girls

Marshall and Tanner's data (Marshall and Tanner 1969) showed that there was considerable variation in the stages of breast development that the girls were in for any given stage of pubic hair and vice versa. Peak height velocity (instantaneous) and menarche were also each reached at different breast and pubic hair stages. The French study of Coste et al (Coste et al. 2002) included a longitudinal study of 50 normal children (25 boys) in whom they evaluated the relationships between growth and pubertal stages.

The following table has been adapted to summarise the findings of both sets of investigators and presents the percentage of girls in each stage of breast

development on reaching each stage of pubic hair, peak height velocity and menarche:

Breast Stages					
Marshall et al:	1	2	3	4	5
PH2	16	49	27	8	0
PH3	3	23	50	24	1
PH4	2	4	43	45	6
PH5	0	1	11	49	39
PHV	0	26	51	23	0
Menarche	0	1	26	62	11
Coste et al:					
PHV	10	40	30	20	0

Table 1.2c, Girls: Percentage in each breast stage on reaching each PH stage, PHV and menarche, Marshall/Tanner and Coste

It is of interest to note that the automated method of identifying a peak of Coste et al (Coste et al. 2002) identified a spurt in only 80% of girls. They also observed that 10% had a spurt when there was no detectable breast development. This is in contrast to Marshall and Tanner and one has to conjecture whether this may be due to the subjectivity in pubertal assessments since it would be difficult to explain physiologically. The distribution of breast stages between the two studies differs and again this may be due to the unreliability of pubertal assessments or population differences or perhaps a secular trend that affects the timing of the beginning of puberty but not menarcheal age. However, in both studies, 70% or more are in stages 2 and 3 when PHV occurs and none were in stage 5. Just over 10% were in stage 5 when menarche happened and in those girls where data on both menarche and PHV existed, menarche always occurred after PHV.

Marshall and Tanner reported correlations for the interrelationship of pubertal events as: age at menarche and age at PHV = 0.91, age at B2 and age at PHV

= 0.82, and age at B2 and age at menarche = 0.64 (Marshall and Tanner 1969).

1.2.3.3 Boys

As in the girls, there was great variation in the stage of genital development for any pubic hair stage and vice versa. From the data of both Marshall and Tanner (Marshall and Tanner 1970) and Coste et al (Coste et al. 2002), the following table summarises the percentage of boys in each stage of genital development for each pubic hair stage and peak height velocity:

Genital Stages					
Marshall et al:	1	2	3	4	5
PH2	1	13	45	41	0
PH3	0	4	17	75	4
PH4	0	0	6	65	29
PH5	0	0	0	10	90
PHV	0	0	2	76	22
Coste et al:					
PHV	0	8	60	28	4

Table 1.2d, Boys: Percentage in each genital stage on reaching each PH stage and PHV, Marshall/Tanner and Coste

Yet again, as in the girls, it is interesting to note that the distribution of stages in which PHV occurs is different. In the data of Marshall and Tanner (Marshall and Tanner 1970), 76% of boys were G4 and 22% G5, in the data of Coste et al (Coste et al. 2002), 60% were G3 and 28% G4. It is not possible to know if this is a population difference, a secular trend effect or whether it represents variability in pubertal assessments.

1.2.3.4 Sexual Dimorphism in the interrelationships between puberty stages and growth

Although there is only about 6 months difference between the start of breast development in the girls and the first signs of genital enlargement in the boys

(Marshall and Tanner 1969; Marshall and Tanner 1970), there appears to be a universal two year difference in the timing of the peak height velocity between the sexes. There is also a marked difference in the timing of the growth spurt in relation to pubertal events. In the girls PHV occurs early in the sequence of pubertal changes, with approximately 70-76% at B2/3 while in the boys PHV occurs late in their physical development with 60-75% of them at G3/4. Although the details of the events of puberty are extremely variable in both sexes the general sequence remains exceedingly constant.

1.2.4 Body Composition during Puberty

1.2.4.1 Methods of assessment

Body mass index (BMI), defined as weight/height^2 (kg/m^2), has long been regarded as a reasonable surrogate of body fat. In 1985, Garrow and Webster (Garrow and Webster 1985) showed that BMI was not a measure of percent body fat but an estimate of fat mass (kg). Other investigators have confirmed a closer relationship of total body fat and BMI than that of percent body fat and BMI (Pietrobelli et al. 1998). Since the confidence limits of both percent body fat and total body fat (the latter to a lesser extent) were found to be wide, the suggestion from these researchers was that BMI is a useful indicator of adiposity on a group level but cannot predict an individual's fatness. BMI has been shown to have a high correlation with body fat and, as is statistically required, a minimal correlation with height (Fung et al. 1990; Deurenberg et al. 1991). More recent work, however, from the Fels Longitudinal Study group has shown that this does not universally hold for children at all ages. Maynard et al (2001) reported that BMI and stature are related in early adolescence in boys although the relationship in girls was less clear (Maynard et al. 2001). Although

BMI does not distinguish between fat mass (FM) and fat free mass (FFM) it is widely accepted as a "reasonable index of adiposity.....and is the preferred measure of adiposity for routine clinical and public health purposes" (Dietz and Robinson 1998). The advantages of using this index are apparent; weight and height are relatively easy, inexpensive and accurate to obtain using simple equipment. In addition, although BMI has high positive correlations with measures of adiposity, it also has positive correlations to fat free mass. Again, using the data from the Fels Study group, Maynard et al observed that annual increases in BMI were primarily due to increases in fat free mass/ht ^{2} until late adolescence and as expected, total body fat/ht^2 was a larger proportion of the BMI increases in girls than boys. Thus, a larger BMI does not necessarily mean greater body fatness. Wells (Wells 2000) emphasized this very clearly with his use of a Hattori chart in infants and children and Prentice and Jebb (Prentice and Jebb 2001) reviewing the literature demonstrated that individuals with the same BMI could have widely differing body fat. Since BMI varies with age and sex across age in children, data are often expressed as BMI standard deviation scores (BMI SDS) for a given age and sex (Cole et al. 1995).

The human body consists of more than 30 different components at the atomic, molecular, cellular, tissue, and whole body levels (Houtkooper 1996). Since the direct measurement by chemical analysis of body composition in human beings is not possible, indirect methods are used. Various methods available for the assessment of body composition include: densitometry, isotope dilution, total body potassium, dual-energy X-ray absorptiometry (DXA), anthropometry and bioelectrical impedance analysis (BIA). The first four are often regarded as 'reference' or 'criterion' methods and the latter two as bedside or field

techniques. They vary in accuracy, complexity, cost and availability; some are impractical, unwieldy or too expensive for field situations and may require equipment and procedures not suitable for use in children. At present there is not a true 'gold standard' for the measurement of body composition in children (Treuth et al. 2001; Mast et al. 2002). All current methods use method and algorithm specific estimates of fat and lean tissues that are based on a number of assumptions. Densitometry and DXA have been used in adults but not extensively in children where in general standard techniques and published algorithms have been employed.

Skinfold measurements and bioelectrical impedance are indirect methods that are easily used in a paediatric population and both have %body fat estimates that are within 3-4% of criterion values (Houtkooper 1996). The results of the measurements are placed in prediction equations that have been validated against direct methods (Chumlea and Guo 2002). These equations are population specific and assume that body density does not change with age or sex. In spite of these assumptions, the FM and FFM estimates obtained correlate well to criterion estimates (Zemel 2002). Two terms are often used in relation to the fat free tissues; one, 'lean body mass' refers to the fat free mass plus 'essential lipids' and the other is 'fat free mass' (FFM) which is the weight of all tissues minus the ether extractable fat and is the term that will be used here.

The simplest estimation of body composition is that based on a two-compartment model and most studies to date have used this (Rolland-Cachera 1993). In this model the body is divided into fat mass (weight of all extractable lipid with ether as the solvent) and the rest are grouped together as the fat free

mass. The assumptions involved are that fat mass is anhydrous and has a density of 0.9g/cm^3 while fat free mass is assumed to have a constant chemical composition and have a density of 1.1g/cm^3 and water content of 72-74% and there is no change with age (Siri 1956). Although there is evidence that this assumption holds for fat mass (Guo et al. 1989) which changes little with age, it is not valid for fat free mass. Lohman (Lohman 1986) has shown fat free mass increases with age since it is dependent on the relative proportion of bone and muscle and therefore varies with age, sex, and maturation as well as state of hydration (young children have greater body water and therefore a lower body density). Density of FFM cannot be measured accurately and is determined only by cadaver analysis; it is a function of the relative amounts of water, protein and minerals (Weststrate and Deurenberg 1989). Whole body cadaver analyses during childhood have not been done (Ellis 2000).

1.2.4.2 Girls and Boys

Although total body weight increases from childhood through puberty to adulthood (the UK reference (Freeman et al. 1995) shows an average increase of 60% from 11 to 18 years in girls and 78% in boys from 12 to 19 years) the different components of body composition vary during this time. There is the well known decrease in BMI during childhood until its nadir around the age of 5 - 6 years before it starts to increase. Fat free mass and fat mass increase steadily in girls whereas percent body fat increases overall but may display a mid-pubertal dip, overall some observers have stated that percent body fat is relatively constant in girls. In the boys, fat free mass increases, fat mass increases then appears to decrease during mid puberty before increasing again

and percent body fat increases, decreases and then increases again. Boys appear to lose fat temporarily and percent body fat progressively decreases. The various methods of assessment of body composition and different study designs give rise to a variety of results which are often reported in different ways. The following tables attempt to summarise some of the literature from the past two decades in both sexes but is by no way exhaustive.

GIRLS	Author	Study	Method	Results
FFM	Buckler 1990	Leeds long, N=102	skinfolds	An increase from 24kg at 10y to 39kg at 17y or an increase of 2.3kg/y from 10-17y
	Cameron,Demerath 2002 Review	Citing Forbes 1987 data		An increase of 16kg from 10 to 20 y
	Guo 1997	Fels long, N=114, 8-23y	hydrodensitometry	Mean increase from 24g at 9y to 44kg at 19y
	Ellis 2000	Cross sec, Caucasian N=292	TBW (D_2O), TBK	Increases from 21.3kg at 8y to 45.1kg at 17y
	Lloyd 1998	Penn State Longitudinal, N=82	DXA	Increases steadily from 11 to 18y
FM	Cameron, Demerath 2002 Review	Citing Roche 95 and Chumlea 83		Increases at an annual rate of 1.1 kg/yr
	Buckler 1990	Leeds long, N=102	skinfolds	Skinfold values increased by 50%
	Guo 1997	Fels, N=114	hydrodensitometry	Mean increases from 6.4kg at 9y to 16.32kg at 19y
	Ellis 2000	Cross sec, Caucasian N=292	TBW (D_2O), TBK and Wt-FFM	Increases from 9kg at 8y to 23.5kg at 16y
	Lloyd 1998	Penn State, 11-18y, Longitudinal, N=82	DXA	Average increase of 6kg from 12-18y fastest velocities before 12y and after 16y
PCBF	Cameron, Demerath 2002 Review	Citing Roche 95 and Chumlea 83		Claim there is a general agreement of little or no change in pcbf
	Schaefer 1998	German cross sec, N=2554, 6-19y	skinfolds	Steady increase with age. By puberty, observed 'dip' at stage 3-4
	Gasser 93,94	Zurich Long	skinfolds	Quoted by Schaefer as also observing this mid-pubertal 'dip'
	Guo 1997	Fels Long	hydrodensitometry	Overall mean increase from 20 to 26% from 9 to 19y
	Lloyd 1998	Penn State Longitudinal, N=82	DXA	Decreases between 13.5 and 16y, greatest increases before 12y and after 16y
	McCarthy 2006	English cross sec,N=869,5-18.5y	bioimpedance	50^{th} c increases from 21.2% at 8y to 24.6% at 18y
	Buckler 1990	Leeds long, N=102	skinfolds	An increase from 19.6% at 10y to 25% at 17y

Table 1.2e, Girls: Summary of selected body composition studies

BOYS	Author	Study	Method	Results
FFM	Buckler 1990	Leeds long, N=96	skinfolds	An increase from 25kg at 10y to 53kg at 18y or an increase of 3.8kg/y from 10 to 17y
	Cameron,Demerath 2002 Review	Citing Forbes 1987 data		Increases by 33kg from 10 to 20y, more rapid between 12-15y
		Citing Roche 95 and Chumlea 83		An annual increase of 4.38kg/y
	Guo 1997	Fels long, N=130, 8-23y	hydrodensitometry	Mean increase from 24.5kg at 9y to 60.2kg at 19y
	Ellis 2000	Cross sectional, total Caucasian N=292	TBW (D_2O), TBK	Increases from 22.7kg at 8y to 61.9kg at 18y
FM	Cameron, Demerath 2002	Citing Roche 96 and Chumlea 83		An almost unchanging fat mass
	Buckler 1990	Leeds long, N=96	skinfolds	Relative constancy throughout pubertal age range
	Guo 1997	Fels long, N=130, 8-23y	hydrodensitometry	An increase from 4.7kg at 9 y to 9.7kg at 19y
	Ellis 2000	Cross sectional, total Caucasian N=292	TBW (D_2O), TBK and Wt-FFM	Increases from 5.5kg at 8y to 20.2 kg at 13y,then decreases to10.7kg at 16y and plateaus
PCBF	Buckler 1990	Leeds long, N=96	skinfolds	Not much change, 17% at 10y, then decreases slightly until 16y and then increases to 18.9% at 18y
	Cameron, Demerath 2002	Citing Roche 95 and Chumlea 83		A decrease of 1.15%/y due to increase in FFM and almost unchanging FM
	Schaefer 1998	German cross sec, N=2554, 6-19y	skinfolds	Increases up to 13y, then decreases followed by a small increase after 16y. Decreases late prepub to G4 and then a slow increase.
	Gasser 93,94	Zurich Long	skinfolds	Schaefer quotes long Zurich study also reports this mid-pubertal dip
	Guo 1997	Fels long, N=130, 8-23y	hydrodensitometry	Increases from 14.9-17.6% from 8-14y, decreases from 17.6-11.4% from 14-18y and then 11.4-13% from18-20y
	McCarthy 2006	English cross sec, N=869, 5-18.5y	bioimpedance	50^{th} c relatively flat from 15.6% at 5y to 15.4% at 18y rising to 17.8 % at 10-11 years

Table 1.2f, Boys: Summary of selected body composition studies

1.2.4.3 Effect of timing of puberty

Whether the age at onset of puberty affects body composition or conversely whether body composition affects the age of pubertal onset has long been an area of research. More than 40 years ago Tanner cited a number of investigators who observed an association between higher weight for height and early maturation (Tanner 1962). The critical weight hypothesis of Frisch and Revelle in the 1970's (Frisch and Revelle 1970) suggested that there was a direct relation between a 'critical weight' (said to be about 48kg) and menarche. They claimed that both early and late maturers would have menarche at the same weight. Johnston et al (Johnston et al. 1971) claimed that using mean weights for menarcheal age groups led to erroneous conclusions and using a multiple regression analysis involving age at menarche, height and weight they refuted Frisch and Revelle's hypothesis. Their findings were based on the fact that height, weight and age at menarche are interrelated and at a constant height, weight and age at menarche are significantly negatively related.

Forbes (Forbes 1992) pointed out that Frisch et al did not perform any body composition analyses but used height and weight to estimate body fat. It is clear from the studies on body composition (see previous tables) that there is a greater increase in lean tissue than fat tissue in both sexes and thus body fat cannot be singled out as the main trigger for the onset of menarche.

It is well recognised that there is a negative relation between BMI and/or body fat and age at menarche (Tanner 1962; Buckler 1990; van Lenthe et al. 1996b) but it remains unclear if early adiposity causes an earlier puberty or if an early puberty leads to an increase in body fat. Although it has been observed that early maturers tend to be heavier than average or late maturers not all overweight girls are early maturers. In fact, de Ridder et al (de Ridder et al.

1992) found that the age of pubertal onset in a group of 68 girls followed for 3 years was not related to fat mass. An Australian study using peak height velocity as the 'pubertal' marker found no relation to the age of PHV and weight or fat mass in either 60 boys or 53 girls studied (Iuliano-Burns et al. 2001) Interestingly, the longitudinal study of Biro et al found that 443 girls out of 859 (51.6%) had an 'asynchronous' pubertal maturation (Biro et al. 2003). They used this term to differentiate between those girls who presented with pubic hair as their first pubertal sign (adrenarche pathway) or those with breast development (thelarche pathway). Their main conclusion was that those girls who entered puberty through the thelarche path had greater percent body fat 1 year before and throughout puberty compared to those who entered through the adrenarche path.

Siervogel et al (Siervogel et al. 2003) analysed menarcheal data from two cohorts in the Fels Longitudinal Study; the first was composed of girls born from 1929 to 1954 and the second group was born between 1955 and 1982. There was no difference in the mean stature or the mean age of menarche between the two cohorts analysed longitudinally by years from menarche. There was, however, a significant difference in BMI from 4 years before menarche to 6 years after. Their conclusions were that in spite of a significantly greater BMI (an indicator of adiposity, albeit imperfect) between the two cohorts, there was no change in the rate of growth or sexual maturation (including age at menarche). They also found that those children who were more pubertally advanced tended to be taller and have a greater percentage of body fat. This confirms our clinical impression stated above that adiposity does affect pubertal development or rate of growth to the extent that puberty affects growth and body composition.

1.2.4.4 Sexual dimorphism

Regardless of the study, the general pattern of body composition change during puberty shows that girls have both greater total body fat and percent body fat than boys and that this difference increases with age between 10 and 18 years. Mean values for height, weight and fat free mass are often not too dissimilar in early puberty but from the age of 14 onwards a growing dichotomy occurs as the boys overtake the girls. In a recent elegant review Wells discusses the sexual dimorphism and says that although it is evident from fetal life it becomes apparent during puberty (Wells 2007).

The weight gain in boys during puberty is primarily due to the great increase in the acquisition of fat free mass and a concomitant decrease of percent body fat. Girls, on the other hand, have a greater increase of fat mass relative to fat free mass. The resulting sexual dimorphism is that young females have 22-24% body fat compared to 16-18% in young males. (Cameron and Demerath 2002). McCarthy et al (McCarthy et al. 2006) state that at 18 years of age girls have proportionately 60% more body fat than boys (24.6% vs 15.4%).

Most of the studies looking at an association of sexual maturity and adiposity have been done in girls as detailed above. Wang (Wang 2002), however, observed that early maturing boys were less likely to be obese (OR=0.4 (0.2-0.8)) than their counterparts whereas early maturing girls were twice as likely (OR=1.96 (1.11-3.47)) to be obese.

1.3 Endocrinology of Growth, Puberty and Body Composition

1.3.1 Adrenarche and the adrenal androgens

Adrenarche has been defined as the "puberty" of the adrenal gland and is characterized by increases in DHEA and DHEAS production from the zona reticularis of the adrenal cortex (Ibanez et al. 2000). These hormones are

generally referred to as the adrenal androgens because they can be converted peripherally to testosterone. They are, however, regarded as androgen precursors since they have negligible binding or activating roles with respect to the androgen receptors.

In the 1940's Albright et al coined the term 'adrenarche' to refer to increased adrenal androgens and the descriptive clinical term 'pubarche', the appearance of pubic hair (Ibanez et al. 2000). Adrenarche occurs between the ages of 6 and 8 years, approximately two years before gonadal maturation. Grumbach and Styne (Grumbach and Styne 2003) have postulated that there may be an unknown pituitary hormone that triggers adrenarche or perhaps some internal adrenal sensing mechanism that initiates it.

A number of studies show that adrenal androgens appear to partially control skeletal maturation. Jääskeläinen and Voutilainen observed accelerated growth in infants and young children with CAH before the initiation of glucocorticoid therapy (Jaaskelainen and Voutilainen 1997). Tall stature and advanced bone age was observed by Ibanez et al (Ibanez et al. 1992) in 127 girls with isolated premature pubarche that was felt to be secondary to increased adrenal androgen secretion and a progressive increase in DHEA and DHEAS paralleled an increase in skeletal age in normal children in several studies (Reiter et al. 1977; Sizonenko and Paunier 1986). Androstenedione (A4), however, does not reflect levels of adrenal production since it can be formed peripherally from DHEAS as well as from the gonads. Although it has been tempting to try and link elevated adrenal hormones at adrenarche to the midchildhood growth spurt and to an initiation of pubertal growth there is no definitive data to support either of these suggestions.

Insulin and leptin have been proposed as triggers for the onset of adrenarche (Biason-Lauber et al. 2000) and Tanner (Tanner 1989) suggested that there might be some relation to the increase in subcutaneous fat at the age of adrenarche. Remer (Remer and Manz 1999) observed that adrenal androgen production was related to increases in BMI regardless of age or sex and in obese women, DePergola et al (De Pergola et al. 1993) found an association between IGF-I and DHEAS. It has since been reported in case control studies that IGF-I and insulin are higher in girls (Ibanez et al. 1997; Silfen et al. 2002) and boys (Denburg et al. 2002) with premature adrenarche than in controls. However, Guercio et al (Guercio et al. 2002; Guercio et al. 2003) observed a sexual dimorphism in a study of 61 normal Argentinean girls and 56 boys from infancy to post puberty in their observational studies of the relationships between BMI, IGF-I, insulin and DHEAS from prepuberty through puberty. A positive correlation between IGF-I and DHEAS in the prepubertal but not the pubertal girls was seen that was not evident in the boys at any stage. Another suggestion has been that insulin or IGF-I increases 17,20 lyase activity and thus promotes adrenal androgen production (Zhang et al. 1995).

Although there have been numerous studies exploring adrenal steroid production in children looking for the trigger for adrenarche, many have been confounded by cross sectional study and often coincident gonadarche with the exception of the studies of Kelnar, Sizoneko and Remer (Sizonenko et al. 1976; Kelnar and Brook 1983; Remer and Manz 1999). Palmert et al studied a group of girls longitudinally who were being treated for central precocious puberty thereby removing possible confounding effects of puberty. They concluded that adrenarche may be the result of a 'progressive maturational event not characterised by abrupt increases in adrenal production'.

1.3.2 Puberty and the Adrenal Androgens

There is not a great deal of evidence that adrenal androgens play much of a role in the onset of puberty or the maturation of the hypothalamic-pituitary-gonadal axis in normal children (Grumbach and Styne 2003). It is of interest to note that although pubic and axillary hair in girls is thought to be under adrenal androgen influence (Sklar et al. 1980), studies using an LHRH analogue have shown that some girls with precocious puberty have a regression of their breast development as well as that of their pubic hair (Neely et al. 1992).

Ibanez et al observed that most children with premature adrenarche enter puberty at a normal age and have menarche within the normal range (Ibanez et al. 1992). Equally children with adrenal insufficiency have been reported to have puberty at a normal age and to have a normal pubertal growth spurt (Grumbach and Styne 2003). On the other hand, Parker (Parker 1991) observed that children with poorly controlled or untreated CAH, had high levels of androgens and entered puberty early. From these results it was postulated that the adrenal androgens might initiate activation of the hypothalamic-pituitary-gonadal axis.

Their role in humans is unclear; however, and although it is not known what triggers the adrenarche process (since there is no change in ACTH or cortisol secretion at this time), it is felt to be independent of gonadarche (increased production of the gonadal sex steroids) (Sklar et al. 1980).

The presence or absence of adrenarche and its affect on the onset of puberty remains controversial.

1.3.3 GnRH Pulse Generator

The medial basal hypothalamus (MBH) contains the LHRH neurosecretory neurons that translate neural signals into an oscillating chemical signal (LHRH). This MBH complex is referred to as the 'LHRH pulse generator' and releases LHRH into the hypothalamic pituitary portal circulation. Thus control of the onset of puberty lies in the central nervous system. Studies of Knobil et al (Wildt et al. 1980) on immature female rhesus monkeys established that pulsatile injections of GnRH was sufficient to initiate ovulatory menstrual cycles despite the immature gonad and pituitary and on cessation of the infusion the animals reverted to their prepubertal status.

After the heightened activity of the hypothalamo-pituitary-gonadal axis during the first six months of life, there is then a period of relative calm before the pubertal avalanche (Conte et al. 1980). In a commentary by Palmert and Boepple (Palmert et al. 2001), they pointed out that the change from a quiescent state of GnRH production in childhood to the adolescent pattern is not an abrupt one but occurs gradually. Citing animal models and human studies they suggest that the hypothalamic neurons are synthesising and secreting GnRH throughout childhood. Pulses of LH and/or FSH have been seen in children as young as 4 years old with the use of highly sensitive immunoassays. The predominant night-time secretion changes to daytime as well and the increased levels are due to an increase in the amplitude and possibly pulse frequency. This latter observation seems to be under debate and may depend on the method used.

Bridges et al (Bridges et al. 1994) studied the pulsatile changes in 24-hour profiles of LH and FSH in children aged 4.2-15.6 years. They observed that children aged 4.2 to 6.9 years of age had higher baseline levels of LH than

those aged 7.1-9.8 years and in both groups pulse frequency was slow. In the older pre-pubertal children, they saw an increase in the mean 24-hour concentrations of both LH and FSH with changes in LH of increasing amplitude (with no baseline change) and increase in LH pulse frequency. They speculated that this increase was similar to that seen in the isolated rat hypothalamus before puberty (Bourguignon and Franchimont 1984) and might trigger the onset of puberty. They did comment, however, that this might also be a response of the hypothalamus to changing concentrations of adrenal androgens at this time. The answer to what initiates the onset of puberty lies in finding the cause of the pubertal increase in the pulsatile secretion of LHRH (which stimulates the pituitary to increase its release of LH and FSH). The mechanism for this is still elusive although in an extensive review, Terasawa and Fernandez (Terasawa and Fernandez 2001) discuss various theories. They reviewed the original 'gonadostat' or 'differential sensitivity to ovarian steroids' hypothesis that was originally proposed more than 50 years ago. This stated that puberty occurred when the regulating system for gonadotrophin secretion became desensitised to steroid feedback as maturation proceeded and this then permitted gonadotrophin secretion. Their suggestion is that the term of 'gonadal steroid dependent LHRH increase' coined by Reiter and Grumbach (Reiter and Grumbach 1982) most accurately describes the gonadostat theory. This suggested that since a smaller amount of gonadal steroids were needed to suppress FSH and LH in prepubertal children compared to adults that a gonadal steroid dependent increase in LHRH occurs at puberty. It would seem that a low level of LHRH maintains a minimum amount of gonadotrophin secretion that is subject to the negative feedback of oestrogen and testosterone. The onset of puberty then is the result of decreasing sensitivity. In a recent review, Veldhuis

et al (Veldhuis et al. 2006) claim that while this theory may be valid in the rodent, it does not hold for the monkey or human and to support this statement cited studies where a pathological lack of sex steroids did not lead to 'castrata-like' high levels of gonadotrophins.

The second hypothesis of 'central inhibition' of GnRH or the 'intrinsic restraint concept' refers to the quiescence of the system before puberty independent of any negative feedback of gonadal steroids, i.e. a central inhibitory restraint on GnRH release. As puberty approaches, this central inhibition is removed or decreased allowing an increase in the amplitude and frequency of GnRH.

1.3.4 Growth Hormone

1.3.4.1 Growth and Puberty

Finkelstein and colleagues did one of the earliest studies on the dynamic secretion of GH in 1972 (Finkelstein et al. 1972). They performed 24-hour overnight studies with 20 minute sampling and demonstrated the episodic release as well as the pubertal increase in the pattern of GH secretion. This pubertal increase has since been substantiated by numerous authors (Miller et al. 1982; Mauras et al. 1987; Blizzard et al. 1989; Martha et al. 1989; Edge et al. 1990). The increase in the secretion of growth hormone happens as a result of a change in the amplitude of the GH pulse whereas its periodicity of 180-220 minutes remains unchanged (Mauras et al. 1987; Hindmarsh et al. 1988b; Blizzard et al. 1989; Edge et al. 1990). Rose et al (Rose et al. 1991) corroborated these findings by performing 24-hour GH profiles, bone ages and pubertal assessments on 132 normal children and adolescents. They found that GH levels increased in girls earlier than boys and that this was most evident at night and that this increase was as a result of an increase in pulse amplitude and not pulse frequency. They divided their prepubertal girls into two groups,

those less than 8 years and those 8-10 years, it is of interest to note that they saw an increase in GH in the latter group before there was any clinical signs of puberty. The earlier pubertal rise in GH in girls compared to boys reflects the close temporal relationship to the adolescent growth spurt.

Numerous studies have shown that the 2-3-fold increase in GH secretion during puberty is paralleled by an acceleration of growth (Hindmarsh et al. 1988b; Stanhope et al. 1988; Edge et al. 1990; Delemarre-van de Waal et al. 2001). In the study of Edge et al (Edge et al. 1990) all measures of pulse height of GH were at their maximum at breast stages 2-3 in the girls and genital stages 4--5 in the boys corresponding to the adolescent growth spurts in each sex.

Veldhuis et al (Veldhuis et al. 2000) addressed the question of sex differences using overnight 12-hour profiles sampling at 10-minute intervals in prepubertal and late adolescent (stage 4 and 5) youngsters. GH pulsatility was observed in both prepubertal and pubertal subjects of both sexes. There was a greatly heightened increase in the secretory burst mass of GH in the adolescents, which the authors were able to break down as due to an increase in both amplitude and duration. The main sex difference was the disorderliness of the GH patterns in the late adolescent girls.

1.3.4.2 Body Composition

An inverse relationship of weight or obesity to GH secretion during puberty has been observed by some researchers (Loche et al. 1987; Martha et al. 1993; Albertsson-Wikland et al. 1994). A more recent report by Roemmich et al explored GH secretory dynamics with 12 hour overnight profiles in lean and overweight subjects both prepubertal and pubertal (Roemmich et al. 2005). Using deconvolution analyses to quantify GH secretory events, they concluded

that there was a lower mean GH concentration in overweight subjects due to a decrease in amplitude with no effect on frequency.

A sex difference in this relationship in normal children was seen by Rose et al (Rose et al. 1991) who observed it in girls but not boys. They used body mass index (BMI) expressed as an SD score and found a significant negative correlation in the girls that was most marked from stages 3 to 5. The greater fat mass that the girls accrue during puberty was felt to be responsible for this sex difference.

The relationship between body fat and GH is seen in growth hormone deficient children and adolescents who often have increased body weight with central adiposity in relation to their height. Treatment with GH has been shown to result in a decrease in fat mass and increased lean mass (Carroll et al. 2004; Gleeson et al. 2007).

1.3.5 Insulin-like Growth Factor I (IGF-I)

1.3.5.1 Growth and Puberty

It has long been accepted that most of the postnatal growth promoting effects of growth hormone are mediated by insulin like growth factor-I (IGF-I) (Salmon and Daughaday 1957; Daughaday 1997; Salmon and Burkhalter 1997). IGF-I, a peptide of 70 amino acids, is produced predominantly by the liver but also by a variety of other tissues where it can act in an autocrine, paracrine or endocrine manner. It has a high degree of structural homology to both proinsulin and insulin and has short-term metabolic effects and long-term growth effects. There are no identifiable tissue stores of IGF-I and although it is produced by a number of different tissues, the liver is the main source. It circulates largely bound to one of the six binding proteins that modulate its activity and this binding protein (IGFBP- 3) binds more than 95% of IGF-I. This dimer forms a

ternary complex with the acid labile subunit (ALS) and acts as a circulating reservoir of IGF-I with a half-life of several hours (Le Roith 1997). IGF-I acts on almost every organ in the body and plays a prominent role in postnatal growth. The actions of IGF-I are mediated through the IGF-I receptor which is structurally similar to the insulin receptor and although IGF-I can bind to the insulin receptor it does so with a lower affinity than insulin (Neely et al. 1991; LeRoith et al. 1994).

In the 1980's, studies by Luna et al, Pescovitz et al, Rubin et al and Cara et al (Luna et al. 1983; Pescovitz et al. 1985; Rubin et al. 1986; Cara et al. 1987) demonstrated that serum levels of IGF-I were low in childhood and rose gradually with a steep increase during puberty. The obvious question was whether this pubertal increase in IGF-I played a role in the pubertal growth spurt. The data, however, is difficult to untangle, Pescovitz et al studying children with central precocious puberty observed that growth velocity levels returned to normal prepubertal growth rates after treatment with a GnRH analogue but that levels of IGF-I were only marginally decreased (Pescovitz et al. 1985). Cara et al, in a small longitudinal study, observed that the peak in IGF-I levels occurred approximately 1 year after peak height velocity (Cara et al. 1987). Juul (Juul et al. 1994) in a large cross sectional study reported that the peak occurred at about 14.5 years in girls and 15.5 years in boys and then slowly returned to prepubertal levels.

Both Juul et al and Lofqvist et al were able to observe an age dependency within pubertal stages and saw a positive relationship between age and IGF-I levels in early puberty although a negative one in late puberty (Juul et al. 1994; Lofqvist et al. 2001). Lofqvist observed a sex difference in midpuberty with the girls showing no age effect while the boys still demonstrated a positive age-IGF-

l relationship. It would appear that the change from a positive relationship to a negative one between IGF-I and age or height velocity occurs since height velocity decreases in mid to late puberty while IGF-I levels are still increasing. Since levels of IGF-I remain high while the growth velocity is decreasing this would appear to support the suggestion for a further role for IGF-I in the continuing post pubertal growth that occurs in cortical bone, muscle and adipose tissue (Cara et al. 1987).

IGF-I levels displayed a significant sex difference (girls >boys) in pubertal children but not prepubertal ones in the data of Juul (Juul et al. 1994). Lofqvist et al examined this more closely by looking at older (>8y) prepubertal children (Lofqvist et al. 2001) where they did observe a sex difference as well as one in those in puberty. The small study (12 boys and 8 girls) by Cara et al (Cara et al. 1987), however, reported no difference in the peak IGF-I values between the sexes.

Studies on pubertal children of both sexes have demonstrated a correlation between IGF-I levels and sex steroids (Juul et al. 1995; Lofqvist et al. 2001; Clayton and Hall 2004). It is known that gonadal steroids increase the pulsatile secretion of GH (Ho et al. 1987) and that GH is correlated to IGF-I. The stimulation of IGF-I by gonadal steroids is thought to be indirect, primarily through increased GH secretion (Harris et al. 1985; Juul et al. 1995). In a study of 10 boys with hypopituitarism, Mauras et al reported an increase in IGF-I levels with testosterone treatment that was increased 3 times with the addition of GH (Mauras et al. 2003). The study of the interaction of sex steroids and the IGF-I/GH axis has been complicated by the fact that sex steroids can act directly on the growth plate. This has been demonstrated in the study of Attie et al on children with central precocious puberty (CPP) and GHD; the results after

treatment with a GnRH analogue showed that although growth velocity decreased, IGF-I levels were not much altered (Attie et al. 1990). However, Juul et al studying children with CPP compared the use of a GnRH analogue and a combination of a GnRH analogue with an anti androgen (cyproterone acetate, CP) (Juul et al. 1995). Although the progress of puberty was halted with the suppression of the gonadotrophins as well as a decrease in growth velocity, they showed no effect on IGF-I levels (in agreement with Attie et al) with the analogue alone although there was an increase in IGFBP-3. This led to the speculation that the decrease in velocity might be due to a decrease in the biologically 'free' IGF-I. Treatment with both the analogue and CP resulted in a decrease of both IGF-I and IGFBP-3. Height velocity was correlated to IGF-I and IGFBP-3 and not E2 in these children with precocious puberty.

High doses of E2 have been shown to decrease IGF-I levels in tall girls by speeding up epiphyseal closure leading to a reduction in final height (Rooman et al. 2005). The results of a study by Coutant et al in children 8-16y exploring the effects of high doses of oestradiol on IGF-I concentrations in 14 girls with delayed puberty observed an inhibition in the responsiveness to GH although they observed the usual increase in IGF-I from pre- to mid-puberty. Thus they reinforced the concept that low doses of oestrogen, as seen in the early stages of puberty, stimulate responsiveness of IGF-I to GH whereas high concentrations could inhibit responsiveness (Coutant et al. 2004). Testosterone priming in 14 delayed boys in this study was seen to not change the responsiveness of IGF-I to GH stimulation.

In elderly adults, Veldhuis et al reported that testosterone increased levels of IGF-I compared to a placebo in men and oestradiol lowered IGF-I levels in postmenopausal women (Veldhuis et al. 2005).

There are obviously factors involved in the modulation of IGF-I other than the sex steroids. In a review by Clayton and Hall (Clayton and Hall 2004) a number of regulators of IGF-I (both general and hormonal) are cited; they include: age, gender, puberty, nutritional status, body composition, IGF binding proteins, GH, testosterone, oestradiol, thyroxine, cortisol and insulin.

1.3.5.2 Body Composition

Nutritional status is an important regulator of IGF-I; subjects with anorexia nervosa and girls, whose intensive training as gymnasts or ballerinas might put them in negative energy balance, have low IGF-I levels. Argente et al found low levels of IGF-I and IGFBP-3 in a group of girls with anorexia and with either high or low levels of GH suggesting that the low IGF-I was due to nutrition and not GH levels (Argente et al. 1997). Both Juul et al and Wilson et al looked at the relationship between body composition and IGF-I levels in healthy normal prepubertal children (Wilson et al. 1991; Juul et al. 1994). Using body mass index (BMI) as a surrogate for body composition, neither found a significant relationship between IGF-I and BMI. Ong et al (Ong et al. 2002), however, employing more sensitive indicators of body composition in a large longitudinal cohort of 497 five year olds observed a significant correlation between IGF-I levels and both fat mass ($p<0.05$) and fat free mass ($p<0.0005$). They also saw an association between IGF-I levels at five years of age and weight gain between 0 and 2 years of age and speculated whether the effects of early nutrition were mediated through IGF-I to affect later growth and maturation.

1.3.6 Insulin

1.3.6.1 Growth and Puberty

Insulin is synthesised in the pancreatic beta cells as proinsulin and then cleaved to form insulin and C peptide (Steiner et al. 1967). It circulates in picomolar concentrations and has a half-life of minutes. Insulin acts on the liver, muscle and adipose tissue.

It has long been known that plasma concentrations of insulin increase with age (Grant 1967) however, it is puberty rather than age that is the key factor in this increase (Lautala et al. 1985; Smith et al. 1988). Fasting insulin levels were seen to be highest in puberty stages 4-5 in the study of Smith et al with no difference between boys and girls (Smith et al. 1988). Insulin is known to have growth promoting effects, Hindmarsh et al observed a linear relationship between height velocity and fasting insulin levels in normal children and noted that the doubling of growth velocity at puberty is accompanied by a 2 to 3 fold increase in insulin (Hindmarsh et al. 1988c).

Amiel et al (Amiel et al. 1986) and Bloch et al (Bloch et al. 1987) independently were the first to demonstrate that it was the hormonal and physical changes of puberty itself that played a role in the insulin resistance seen in adolescents. Using the euglycaemic hyperinsulinaemic clamp technique they showed a reduction in insulin mediated glucose metabolism during normal puberty compared to prepubertal children and adults. Bloch had also observed an increased insulin response to an oral glucose tolerance test in pubertal children compared to prepubertal ones.

To assess the effects of insulin on protein metabolism, Amiel and Caprio et al (Amiel et al. 1991) used the euglycaemic hyperinsulinaemic clamp technique and observed that the insulin resistance selectively affected peripheral glucose

metabolism, there was no resistance to the anabolic effects of insulin. Confirming this a few years later, Caprio et al using an infusion of labelled leucine also found a reduction in insulin stimulated glucose uptake in pubertal compared to pre pubertal children and adults but observed no effect of this normal pubertal insulin resistance on insulin stimulated protein metabolism (Caprio et al. 1994).

The compensatory increase in insulin secretion, that maintains glucose homeostasis during puberty, in the face of the insulin resistance, was demonstrated by the hyperglycaemic clamp technique (Caprio et al. 1989). Caprio et al showed that there was a 2-3 times increase in the insulin responses in pubertal subjects compared to adults (Caprio 1999). They corroborated the earlier conclusions of Holly (Holly et al. 1989) that the insulin resistance of puberty results in a compensatory hyperinsulinaemia and thus a decrease in IGFBP-1 levels that in turn increases the bioavailability of free IGF-I and thus insulin modulates pubertal growth.

1.3.6.2 GH and Insulin sensitivity

These pubertal changes occur at the same time as the pubertal increase in growth hormone and the antagonistic effects of growth hormone on insulin are well known (Rizza et al. 1982). A number of studies have suggested a causal relation of the rise of growth hormone and the insulin resistance and hyperinsulinaemia of puberty (Amiel et al. 1986; Smith et al. 1988; Edge et al. 1990; Savage et al. 1992). Amiel et al observed that the response to insulin during a euglycaemic insulin clamp was inversely related to the 24 hour GH level but not with the level of IGF-I in all the children (Amiel et al. 1986; Amiel et al. 1991). Changes in insulin concentrations during puberty and their relationship to growth hormone were studied by Hindmarsh et al who saw the

highest levels of insulin in those children who had the highest growth hormone levels on 24 hour testing (Hindmarsh et al. 1988a). Although it seems contradictory that at a time of rapid growth there should be an antagonistic conflict between these two anabolic hormones, it has been suggested that the compensatory hyperinsulinaemia may enhance the anabolic effects of GH, IGF-I and sex steroids on protein metabolism during pubertal growth (Mauras et al. 1996).

1.3.6.3 Insulin Resistance of Puberty

In a large cross sectional study on 357 normal children aged 10-14 years of age, (encompassing all five puberty stages), Moran et al (Moran et al. 1999), using the euglycaemic clamp technique, reiterated that insulin resistance was a transient event of normal puberty. They observed an increase in resistance at the beginning of puberty that peaked at stage 3 in both sexes and returned to near prepubertal levels by stage 5. Girls were more insulin resistant at all stages than boys and insulin resistance was strongly associated with BMI, triceps skinfold measurement and waist circumference. This relationship was independent of sex or pubertal stage; however, the sex difference was only partially explained by differences in adiposity. Insulin resistance was related to both BMI and adiposity in each of the pubertal stages but factors other than body composition were felt also to be part of the explanation of the insulin resistance of puberty.

Other hormonal influences (in addition to the previously mentioned GH) on insulin and insulin resistance during puberty have been addressed by a number of investigators. Smith et al found a positive correlation between IGF-I and insulin throughout childhood and puberty in normal subjects (Smith et al. 1989). They conjectured whether this relationship was related to changes in the

pulsatile release of growth hormone. Independently of growth hormone, however, IGF-I and insulin and their receptors have a high degree of sequence homology (Neely et al. 1991; LeRoith et al. 1994).

1.3.6.4 Insulin resistance and IGF-I

Caprio et al were the first to suggest that there might be a relation between the insulin resistance of puberty and IGF-I levels (Caprio et al. 1989). Moran et al on their large number of children who had euglycaemic clamps were able to investigate this across the whole pubertal range on 342 subjects (Moran et al. 2002). They found that the pattern of change of IGF-I was similar to that of insulin resistance during puberty. They found an association of IGF-I with fasting insulin that was different between the sexes; was related to adiposity in the girls but not the boys, and was stronger in the thinnest girls. When the relation between insulin and insulin resistance (as assessed by the clamp method) was examined it was observed that a significant relation existed between the two in both sexes and it was independent of adiposity (although hyperinsulinaemia was dependent on body fat).

The longitudinal study of Hoffman showed insulin sensitivity greater in pre and early pubertal boys compared to girls and the girls compensated with higher insulin responses (Hoffman et al. 2000).

1.3.6.5 Body Composition

BMI and body fat increase during puberty and there are numerous studies to show that body fat is associated with insulin resistance during puberty (Bloch et al. 1987; Cook et al. 1993; Travers et al. 1995; Arslanian and Suprasongsin 1996; Caprio et al. 1996; Gower et al. 1999; Travers et al. 2002). This may only be part of the picture, however, since Cook et al observed that insulin resistance

could occur in puberty without an increase in BMI and it has been observed that young adult women, who may have more body fat than pubertal girls, are more insulin sensitive (Amiel et al. 1986; Caprio et al. 1989). It appears, however, that there may be a different relationship of body composition to insulin operating in children compared to adults. This relates to the question of whether insulin resistance plays a causal role or is a consequence of fat gain. Studies on 2 groups of children favoured the theory that hyperinsulinaemia and insulin resistance favoured fat gain in childhood and puberty but opposed it in adults (Maffeis et al. 2002). In one longitudinal study of 137 children (mean baseline age of 8.1 years) followed for 3-6 years and seen annually by Johnson et al (Johnson et al. 2001), changes in body fat mass were associated with changes in insulin parameters over time. An increase in total fat mass of 15.6% / year that was not influenced by sex or puberty stage was observed and both initial fasting insulin and the acute insulin response to an IVGTT had a positive effect and insulin sensitivity (assessed by Bergman's 'minimal model') a negative effect on the increase in fat mass. Thus, high fasting levels of insulin and a high acute insulin response plus low insulin sensitivity were associated with increased rates of fat gain during early puberty. This study was in some contradiction to the generally regarded view (Rico et al. 1993; Travers et al. 1995; Moran et al. 1999) that girls gain more fat mass during puberty than boys, have a higher fat to lean ratio and generally a higher insulin resistance (partially explained by the adiposity difference).

1.3.6.6 Androgens/ Sex Steroids

Bloch observed a negative relation between insulin sensitivity and DHEAS and thought that adrenarche might be an early predictor of insulin sensitivity (Bloch et al. 1987). Smith et al found a positive relationship between DHEAS and

fasting and stimulated insulin levels in the combined pre and pubertal groups; however, in the prepubertal group assessed alone they found no relationship and concluded that adrenarche is not associated with increased insulin levels before puberty (Smith et al. 1989). Caprio et al did not find any relation between DHEAS and insulin response to the hyperglycaemic clamp causing them to conclude that a direct effect of sex steroids was unlikely (Caprio et al. 1989). Wickman et al when treating a group of boys with constitutional delay of puberty with testosterone and an aromatase inhibitor (which prevents the conversion of androgens to oestrogens) observed a decrease in insulin, a five-fold increase in testosterone (over placebo group), and no change in 17-ß oestradiol or IGF-I. This contrasted with the results in a second group who were treated only with testosterone and had no change in insulin levels but an increase in the concentrations of 17-ß oestradiol and IGF-I. They found that the changes over 12 and 18 months in insulin and IGF-I were correlated. They concluded that this demonstrated that androgens had no direct effect on insulin sensitivity in early-mid puberty (Wickman et al. 2002). In an earlier study of delayed boys, Arslanian et al had also seen no deterioration of insulin action with testosterone treatment (Arslanian and Suprasongsin 1997). To try and tease out the effect of sex steroids on insulin sensitivity without the influence of growth hormone, Saad et al used dihydrotestosterone (DHT) in boys with delayed puberty (Saad et al. 2001). DHT is a nonaromatizable androgen that has been shown to increase height velocity without an increase in growth hormone (Keenan et al. 1993; Eakman et al. 1996). Ten boys were studied for 4 months before and after treatment with DHT: height, weight and fat free mass increased while percentage body fat decreased but there was no change in insulin stimulated

glucose metabolism. Again the conclusion here is that insulin resistance during puberty is not attributed to gonadal sex steroids in boys.

1.3.7 Sex Hormone Binding Globulin (SHBG)

1.3.7.1 Description

SHBG is a circulating glycoprotein synthesized in the liver, with a high binding affinity to both 17-ß oestradiol and testosterone, whose biological function is the transport of these two sex steroids in the circulation. Less than 2% of sex steroids are 'free' in the circulation, the rest are bound to carrier proteins the most abundant of which are albumin and SHBG. About 44% of testosterone in adult males and 66% in women is bound to SHBG, whereas 20% of oestradiol in men and 37% in women is bound to SHBG (Bolton 1984).

1.3.7.2 Puberty and Sex Steroids

During puberty a decrease in the levels of SHBG has been observed in both sexes (Bartsch et al. 1980; Apter et al. 1984; Maruyama et al. 1987; Holly et al. 1989; Blogowska et al. 2003) at a time when there is a concomitant rise in oestrogen and testosterone. Although it has generally been shown that a degree of regulation of SHBG appears to be by sex steroids, with oestrogens having a stimulating effect (Kalme et al. 1999) and androgens an inhibiting one (Anderson 1974) this is not universally observed and there are differences reported between in vivo and in vitro studies.

In a cross sectional study of 69 adolescents, Holly et al observed a reduction in SHBG that was inversely correlated to the increase in testosterone, DHEAS and androstenedione in the boys although only to DHEAS levels in the girls while no relationship between SHBG and oestrogen was found in either sex (Holly et al. 1989). In a more recent and larger study (903 subjects) Sorensen et al

confirmed the negative association of SHBG with testosterone and DHEAS but also with oestradiol as well in boys and only with DHEAS in girls (Sorensen et al. 2007). Kim et al, in a study of 77 boys 10-18years old, saw a decrease in SHBG from puberty stage 1 to 2 then a slight increase which plateaued through to stage 5 (Kim et al. 1999).

Although sex steroids are involved in the regulation of SHBG, it can be seen from the above studies that although there were increasing levels of sex steroids they did not in themselves determine SHBG levels. Of the other factors involved, it has been shown that thyroid hormone (Yosha et al. 1984); insulin and IGF-I play vital roles as well.

1.3.7.3 Relation to Insulin and IGF-I

Strong negative correlations have been found to exist between the falling SHBG levels and rising insulin concentrations in both sexes during puberty as well as a negative relationship between SHBG and IGF-I in boys (Holly et al. 1989). Singh et al 1990 using cultured human hepatoma cells confirmed the inhibitory effect of both insulin and IGF-I on SHBG (Singh et al. 1990). This was thought to show the effect of dietary factors as mediated through IGF-I on SHBG. This may tie in with the observations by Kim et al on the interrelationships of BMI, insulin and SHBG during puberty. They suggested that the inverse association of SHBG and BMI from puberty stage 1 to 3 might play an important role in the regulation of SHBG during early puberty via body fatness and thus insulin sensitivity (Kim et al. 1999).

Kalme et al (Kalme et al. 2003) using human hepatoma cells (which secrete SHBG) demonstrated that insulin inhibited production of SHBG at 48 hours by about 35% whereas IGF-I reduced the levels of SHBG levels after only 6 hours incubation by 24%. An earlier study in 1995 by Loukovaara et al also produced

a reduction by insulin after 48 hours incubation that resulted in a greater effect than IGF-I in lowering the levels of SHBG (Loukovaara et al. 1995). An in vivo study by Strain et al during weight loss in men found that the relationship between insulin and SHBG was remarkably constant despite the changes in the individual relationships of insulin and BMI to SHBG. They concluded that the inverse association points to the fact that insulin controls SHBG in man (Strain et al. 1994).

Several researchers have investigated the suggestion that SHBG could be a marker for hyperinsulinaemia. Most relevant to us is the work of Galloway et al (Galloway et al. 2001) looking at prepubertal children to see if SHBG could be a predictor of hyperinsulinaemia and thus target vulnerable children who may be at risk for premature onset of the metabolic syndrome and consequent cardiovascular problems.

1.3.7.4 Body Composition

Sorensen et al (Sorensen et al. 2007) in a large cross sectional study of 903 children confirmed many previous reports of a negative correlation of SHBG and measures of body composition. This negative relationship remained even after adjusting for testosterone, oestradiol and DHEAS.

Kim et al found an inverse relationship of BMI and SHBG in boys in early –mid puberty (stages 1 to 3) (Kim et al. 1999). This is at a time when the insulin resistance of puberty is high and lends support to the theory that body fat, through its effect on insulin sensitivity, has a role in the regulation of SHBG in early puberty.

1.3.8 Leptin

1.3.8.1 Description

Zhang et al working with the ob/ob mouse discovered the gene encoding for the 16-kda peptide, leptin, secreted by adipocytes and thought to act primarily through specific receptors at the level of the hypothalamus (Zhang et al. 1994). In the mouse, leptin has effects on appetite, energy expenditure and the neuro-endocrine axis (Maffei et al. 1995; Stephens et al. 1995; Vaisse et al. 1996). In humans, congenital leptin deficiency and mutations in the human leptin receptor gene result in severe obesity (Montague et al. 1997; Clement et al. 1998; Strobel et al. 1998). It is unknown if the normal leptin level variation merely reflects fat mass or determines future fat mass accumulation (Qureshi and Kopelman 1997). Longitudinal study of Japanese Americans indicated that higher baseline plasma leptin levels in a study of Japanese Americans were associated with fat accumulation (Chessler et al. 1998) whereas studies in the Pima Indians observed that low leptin levels predicted subsequent weight gain (Ravussin et al. 1997).

1.3.8.2 Puberty

Work in mice and rats led to the hypothesis that leptin could have a role in pubertal development (Ahima et al. 1997; Chehab et al. 1997; Cheung et al. 1997). Other animal studies also indicate that leptin signalling results in interaction with hypothalamic neurotransmitters, principally neuropeptide Y (Stephens et al. 1995; Rohner-Jeanrenaud et al. 1996), and this may influence gonadotrophin secretion. In the human subjects with leptin deficiency or receptor mutations, there was no initiation of puberty or establishment of secondary sexual characteristics (Clement et al. 1998; Strobel et al. 1998). It is not clear whether the accumulation of fat mass, leading to a permissive leptin

signal, is required for the initiation of puberty or whether dynamic short term changes in leptin levels result in pubertal development (Apter 1997).

Leptin levels in pre-pubertal children were no different in boys and girls (Blum et al. 1997; Carlsson et al. 1997; Clayton et al. 1997; Garcia-Mayor et al. 1997). Clayton et al. reported a gradual increase in leptin concentrations in pre-pubertal children aged 5-9 years. A longitudinal study in 8 boys by Mantzoros et al suggested that there might be a brief pulse of leptin preceding the onset of puberty this however, has not been observed in other studies (Mantzoros et al. 1997). A sexual dimorphism in leptin levels occurring during puberty with an increase in girls and a decrease in boys has been observed in a number of cross sectional studies (Blum et al. 1997; Carlsson et al. 1997; Clayton et al. 1997; Garcia-Mayor et al. 1997). These differences may be partly explained by differences in body mass index or hormone levels (Blum et al. 1997; Wabitsch et al. 1997). Unfortunately better estimates of fat mass were only available in a minority of the subjects studied and the cross-sectional design of most of the studies would not allow a true picture of associations with puberty stage and pubertal hormone levels.

An early puberty or menarcheal age has long been associated with greater gains in weight during childhood (Stark et al. 1989; Beunen et al. 1994; van Lenthe et al. 1996a; Wattigney et al. 1999; Adair 2001; Cameron and Demerath 2002; Tam et al. 2006). The original proposal of Frisch and Revelle (Frisch and Revelle 1971) that a 'critical weight' (48kg) is needed for the initiation of puberty provoked much controversy. Delemarre-van de Waal (2002) quoting Ruf from 1973: "how can the brain be informed of the nutritional state of the organism and how can it 'know' when to initiate puberty" (Delemarre-van de Waal 2002). Twenty years later the discovery of leptin gave credence to the idea of a

'sensor' of body fat levels that feeds back to the hypothalamus to activate central gonadotrophin secretion. Studies of leptin replacement for congenital leptin deficiency (Strobel et al. 1998) and in several animal models, Cheung et al (Cheung et al. 1997) suggest that leptin is the permissive signal. However, although overweight children tend to have an earlier puberty, they do not invariably have precocious puberty and so the permissive effect of leptin must also be linked to other maturational events in the hypothalamus.

1.3.8.3 Body Composition

The characteristic sex-specific changes in body composition where gains in fat mass are greater in girls and gains in fat free mass greater in boys that becomes obvious at puberty has been long known (Forbes 1978). In the longitudinal study of Ahmed et al, these differences in body composition during puberty were also observed (Ahmed et al. 1999). The pubertal divergence in leptin levels between the sexes may be attributed to the differences in this acquisition of adult body composition (Blum et al. 1997; Carlsson et al. 1997; Clayton et al. 1997; Garcia-Mayor et al. 1997). Ahmed et al (Ahmed et al. 1999) observed similar relations between leptin and body composition in each sex; allowing for fat free mass, fat mass was positively related to leptin and allowing for fat mass, fat free mass negatively related to leptin levels. Together, fat mass and fat free mass accounted for 54% of the variance in leptin levels in boys, and 31% in girls. Fat mass is a major source of circulating leptin (Zhang et al. 1994) and thus a close relationship with leptin levels is expected, however the negative relationship with fat free mass is unexplained and may relate to parallel effects of other hormones on both leptin production and the acquisition of fat free mass.

1.3.9 Sex Steroids

1.3.9.1 Testosterone

1.3.9.1.1 Description

Testosterone is produced by the Leydig cells of the testes and is the main sex steroid of male sexual development. A small amount is also derived from conversion of androstenedione secreted by the testes and adrenals. In girls, almost all the circulating testosterone is from the conversion of ovarian and adrenal androstenedione. Prepubertal children of both sexes have undetectable levels of plasma testosterone although a diurnal rhythm has been shown to exist in boys as young as 4-5 years old (Mitamura et al. 1999), well before any pubertal signs. Detectable increases in daytime testosterone levels in boys usually begin about the age of 11 years corresponding to a testis volume of 4 mls and then continue to increase throughout puberty. In girls, Ilondo et al (Ilondo et al. 1982) observed a peak and plateau of testosterone in girls at stage 3 (pubic hair).

1.3.9.1.2 Growth and Puberty

It has long been thought that testosterone was the main hormone in boys responsible for acting in concert with GH during the pubertal growth spurt and for fusion of the epiphyses. Testosterone administration to prepubertal subjects has been shown to increase GH secretion (Illig and Prader 1970; Martin et al. 1979). Preece and colleagues (Preece et al. 1984) in a longitudinal study of male puberty found a significant relationship of height velocity and levels of testosterone in puberty stages 1-3 and a negative one in stages 4 and 5. The most dramatic increase in testosterone was observed to be around the year of the pubertal growth spurt suggesting the importance of testosterone in this event. Zemel and Katz (Zemel and Katz 1986) in a three year mixed

longitudinal study of 181 boys observed similar relationships. Delemarre-Van de Waal et al in two short longitudinal study over one year observed an increase in testosterone levels in boys from stage 2 and then an even greater increase from stage 3 to 4 corresponding to the timing of the growth spurt (Delemarre-van de Waal et al. 2001).

Testosterone increases growth hormone secretion by an increase in pulse amplitude (or area under the curve) (Link et al. 1986; Martha et al. 1989) and also acts directly on the growth plate (Corvol et al. 1987). Keenan et al showed that whereas testosterone increased growth hormone (by its aromatization to E2) and growth velocity in short boys with constitutional delay; DHT, a metabolite of testosterone and a non-aromatizable androgen, also accelerated growth velocity in the face of a 50% decrease of GH and it was felt that this effect was directly on the epiphyseal chondrocyte (Keenan et al. 1993).

In order to achieve an optimal pubertal growth spurt, it is necessary to have normal levels of testosterone. Untreated hypogonadal subjects (Uriarte et al. 1992), extremely late maturing boys (Hagg and Taranger 1991) and boys with Klinefelters syndrome (Smals et al. 1974) have only a small growth spurt although they eventually attain a normal or even tall stature. In the absence of gonadal steroids, a taller stature may be achieved since epiphyseal closure will be delayed. In studies where growth was compared in children with both GH and gonadotrophin deficiency compared to those with just GH deficiency, it was observed that in the first group the double deficiency gave better growth results (Burns et al. 1981; Hibi et al. 1989). Current research and clinical practise has included treatment of children with a GnRH analogue to try and reverse puberty to increase growth and in some instances combining this with GH although controversy exists about the efficacy of this treatment (Carel et al. 2004).

1.3.9.1.3 Body Composition

The increase in lean body mass that occurs in boys during puberty is a direct consequence of increasing levels of testosterone. Direct evidence comes from clinical work with delayed puberty; Arslanian and Suprasongsin gave low dose testosterone for four months to boys with delayed puberty that led to an increase in fat free mass and a decrease in fat mass (Arslanian and Suprasongsin 1997). Other clinical examples to support this include (1) studies on replacement doses of testosterone given to hypogonadal men result in an increase in fat free mass as well as muscle size and strength (Bhasin et al. 1997), (2) studies in women with CAH or those given exogenous androgens develop a more male – like fat pattern with greater central fat distribution, and (3) women with androgen producing tumours develop a virilised body composition with an increase in lean mass (Rosenbaum and Leibel 1999).

1.3.9.2 Oestrogen

1.3.9.2.1 Description

Oestradiol is the principal oestrogen produced by ovarian granulosa cells in females and circulates bound to SHBG. Estrone is the next most abundant and results from conversion from androstenedione. In males, the testes secrete a small amount of oestradiol but most of the oestrogen in males comes from peripheral conversion (mainly in adipose tissue) of testosterone to oestradiol and androstenedione to estrone.

Oestrogen has long been known to be the main sex steroid in the female responsible for pubertal growth, sexual maturation, skeletal maturation and acquisition of peak bone mass. It had always been thought that testosterone fulfilled these roles in males.

1.3.9.2.2 Growth and Puberty

Oestrogen promotes linear growth in children and in vitro, Corvol (Corvol et al. 1987) demonstrated that both sex steroids exerted growth-promoting effects directly at the growth plate. It has more recently been realised that oestrogen is the most important hormone for determining final height by its role on epiphyseal closure.

Evidence of oestrogen's role in growth came with the identification in 1994 of a man with an oestrogen receptor mutation causing oestrogen resistance that meant he was unable to respond to circulating levels of oestrogen. He was 204cm tall, had open epiphyses, a bone age of 15 years, was still growing and could not remember having had a pubertal growth spurt (Smith et al. 1994). A year later a case of aromatase deficiency was described with a similar phenotype (Morishima et al. 1995). In this case, a mutation in the P 450 aromatase enzyme that converts androgens to oestrogens was discovered. Treatment with oestrogens closed his epiphyses and stopped growth (Bilezikian et al. 1998).

Several authors have shown that it is the aromatisation of testosterone to oestrogen in boys and oestrogen itself in girls that is the sex hormone stimulus for the increasing secretion of GH at puberty as evidenced by an increase in GH pulse amplitude (Keenan et al. 1993; Eakman et al. 1996; Veldhuis et al. 1997). Early evidence for the role of oestrogen in the pubertal growth spurt and on epiphyseal fusion came from the study by Zachmann et al (Zachmann et al. 1986). In this study of 8 phenotypic females with complete androgen insensitivity (AIS: XY genotype and normal female external genitalia as a result of aromatisation of androgens to oestrogens) the girls were observed to have peak height velocities with magnitudes and timings similar to normal girls. Their

bone ages corresponded better to the male standards and their final heights were closer to the mean final height of normal men not women. In one patient who underwent a gonadectomy and oestrogen replacement, a higher than normal growth spurt was observed but a lower final height than those who did not have their gonads removed.

Delemarre van de Waal et al observed a significant association of oestradiol levels to height velocity in girls in puberty stages 1-3 however, in stages 4 and 5, when velocity was decreasing, oestradiol levels continued to increase. In boys they observed an increase of oestradiol during the later stages of puberty consistent with the timing of the pubertal growth spurt (Delemarre-van de Waal et al. 2001).

1.3.9.2.3 Body Composition

Pubertal increases in oestrogen production results in the greater accumulation of fat in girls compared to boys. Although a difference in body composition has been reported in prepubertal children (Taylor et al. 1997; Mast et al. 1998), this sexual dimorphism in body composition is seen particularly after menarche. In the study by Legro et al percent body fat (by both DXA and skinfolds) showed no appreciable increase from 2 years before menarche until at least 1 year after at which time an increase as assessed by DXA became apparent. This was in spite of exponential increases in urinary estradiol from two years before menarche (Legro et al. 2000). An interesting study by deRidder et al observed that there was no evidence that body fat mass triggers the onset of puberty but rather that body fat was negatively associated with the rate of pubertal development from the onset to menarche. They found that the girls with the later onset of puberty had higher levels of oestrogen and moved faster from B2 to menarche, i.e. that the levels of hormones at the onset of puberty were

predictive of the rate of development. No hormonal differences were observed between the girls in the lowest or highest quartile of skinfold distribution. (de Ridder et al. 1992). Clinical support for the fat promoting effects of oestrogen come from the observation that genetic males with AIS have a female fat distribution whereas postmenopausal women develop a more male like habitus with an increase in the visceral adipose site and subsequent increase in the waist-hip ratio (Rosenbaum and Leibel 1999).

1.3.9.3 Sex differences

In 1988, Stanhope and Brook (Stanhope and Brook 1988) conjectured that girls are more sensitive to changes in GnRH concentrations than boys and hence their slightly earlier entrance into puberty. They attributed the difference in the timing of the growth spurt to the fact that only low concentrations of oestradiol are needed to affect GH levels compared to higher levels of testosterone for the same effect.

Although data had suggested that the prepubertal ovary secreted oestrogen, it wasn't until the ultra sensitive assay of Klein et al (Klein et al. 1994) that this has been possible to observe. A greater bioactivity in prepubertal girls compared to boys may explain the earlier pubertal timing in girls.

1.3.10 Thyroxine

Thyroxine is a 'permissive' hormone, essential for normal pubertal growth and development. In cases of deficiency, whether congenital or acquired, a child's growth slows, skeletal and dental maturation is delayed and weight gain increases (Hernandez-Cassis et al. 1995). The longitudinal study of thyroid hormones during puberty by Dunger et al in children 9.5-15.5 years showed that levels of free T4 fell by 16% from 10 years to a nadir at 12.5 years in girls

(puberty stage 3-4) then increased to a maximum by 15 years, in the boys a similar pattern, although a less marked fall (a decrease of 12%) was observed with the nadir again at puberty stages 3-4 (at age 13.5 years) and rose by 15 years but did not return to baseline levels (Dunger et al. 1990). Total T4 levels followed a similar pattern in the girls although the nadir was slightly later at stage 4 before it rose again. In the boys, however, the prepubertal levels were lower and the fall was again less marked and the nadir occurred at age 4-5 again slightly later than the free T4 and did not return to the early puberty levels. Since IGF-I and IGFBP-3 levels are dependent on adequate thyroid hormone levels (Miell et al. 1993) either directly or indirectly through effects on GH secretion (Nanto-Salonen et al. 1993) it can be seen that adequate levels need to be maintained for normal growth and development.

1.4 Growth and Pubertal Development in Type I Diabetes Mellitus (T1D)

1.4.1 Introduction

Glycaemic control in children with T1D often deteriorates during puberty and poor growth could be one manifestation of this (Ahmed et al. 1998). The teenagers in the Diabetes Control and Complications Trial (DCCT) had higher HbA1c levels than the adults (Drash 1993). Yet it has proved to be difficult to identify a clear relationship between glycaemic control, as judged by HbA1c, and growth in children and adolescents with T1D. Two studies have reported a relationship (Wise et al. 1992; Pitukcheewanont et al. 1995) although there have been other studies where no association could be observed (Jivani and Rayner 1973; Salardi et al. 1987; Thon et al. 1992). It is unclear why some children with 'good' control grow poorly and others with poor control seem to grow normally.

It is well known that abnormalities of the GH/IGF-I axis exist, particularly during puberty in T1D, and could account for some of the poor growth observed. However, levels of IGF-I are low in both boys and girls (Ahmed et al. 1998) yet it is only in the girls that a marked blunting of the pubertal growth spurt is observed (Brown et al. 1994). Despite high levels of GH (Edge et al. 1990; Miller et al. 1992; Pal et al. 1992) levels of IGF-I and IGFBP-3 tend to be low (Batch et al. 1991; Clayton et al. 1994; Strasser-Vogel et al. 1995). Portal insulin insufficiency, growth hormone receptor resistance (as measured by levels of GHBP which is identical to the extra cellular domain of the GH receptor) and therefore low levels of IGF-I may be the cause of this dysregulation. These abnormalities may exist since insulin is a significant factor in the regulation of the GH/IGF-I axis; insulin increases hepatic IGF-I production either by regulation of the GH receptor or by effects on post receptor events (Baxter et al. 1980; Maes et al. 1986). Insulin and IGFBP-1 vary inversely with each other with insulin having an inhibitory effect on IGFBP-1 (Holly et al. 1988) and it has been reported that IGFBP-1 is an inhibitor of IGF-I bioactivity (Taylor et al. 1990; Bereket et al. 1996). The reduced circulating IGF-I levels and reduced IGF bioactivity observed (Taylor et al. 1988) may be a product of the high levels of IGFBP-1 in pubertal children with T1D. One large cross-sectional study failed to observe any direct correlations between IGF-I levels and growth velocity (Strasser-Vogel et al. 1995), however since growth velocity needs a longitudinal study, this result may be misleading.

It would seem that by intensifying insulin therapy, the abnormalities should be corrected but this does not appear to be the case. Although intensive insulin therapy increases IGF-I levels (Dunger and Cheetham 1996), it is not perfect

and it appears to be the levels in the portal vein not the peripheral circulation that determine IGF-I, IGFBP-1 and bio availability in T1D (Brismar et al. 1994).

1.4.2 Growth

1.4.2.1 Height at diagnosis

A number of studies spanning twenty years have presented data demonstrating that children with T1D are taller at diagnosis than control children (Jivani and Rayner 1973; Drayer 1974; Edelsten et al. 1981; Hjelt et al. 1983; Lee et al. 1984; Songer et al. 1986; Salardi et al. 1987; Price and Burden 1992). This observation, however, is not universal and has been contested by other studies (Evans et al. 1972; Tattersall and Pyke 1973; Petersen et al. 1978; Hoskins et al. 1985; Emmerson and Savage 1988). It may be that these different observations have arisen due to the use of an inappropriate reference. If the controls were not contemporary and did not reflect any secular trends that may have occurred since the reference standards were established, the T1D children might appear to be taller when in fact if more appropriate data were used this would not be the case. In the 1994 paper of Brown et al, the height SD scores (SDS) in 184 T1D children were calculated using the 1966 UK standards of Tanner et al and all the children were taller, however, when contemporary local population standards were used only those children diagnosed between 5 and 10 years of age were taller (Brown et al. 1994). Height at diagnosis continues to produce controversial results even in later studies. Several investigators (Blom et al. 1992; Price and Burden 1992; Holl et al. 1994; Ahmed et al. 1998; Bognetti et al. 1998; Holl et al. 1998a; Scheffer-Marinus et al. 1999) have reported taller stature at diagnosis. Huang et al (Huang et al. 2001) divided their Chinese cohort into pubertal and prepubertal (admittedly based arbitrarily on the average pubertal onset age for Chinese children) and found that only the

pubertal girls had a significantly elevated height SDS at diagnosis. Lebl et al (Lebl et al. 2003) in a review of 587 T1D patients born in Vienna and Prague from 1962 -1993 (and diagnosed at median ages of 7.9y in boys and 8.4y in girls) with a similar genetic background found the girls' mean height SDS at diagnosis to be + 0.74 ± 1.46, $p<0.01$ and boys was + 0.15 ± 1.1, $p=0.02$. Luna and colleagues (Luna et al. 2004) observed in a group of 83 newly diagnosed Spanish children that girls had a height SDS of +0.40 whereas the boys' height SDS was only +0.08. Whereas Thon (Thon et al. 1992) and Cianfarani (Cianfarani et al. 2000) in different European populations found those children with diabetes were of equal height at diagnosis to healthy controls.

The age of diagnosis may also be a factor in the relative height at diagnosis. Both Brown and Songer observed that those children diagnosed between 5 and 10 were taller at diagnosis, those over 10 were of similar height to the population and in Brown's sample those diagnosed under 5 years of age were shorter, however, it was noted that they did have short parents (Songer et al. 1986; Brown et al. 1994).

Different patterns of growth of children before the diagnosis of T1D may reflect the genetic diversity in this disease. The Pittsburg USA study group (Anonymous 1989) reported striking differences in their children compared to a comparable group from Japan while Ramachandran et al reported normal heights at diagnosis in southern India (Ramachandran et al. 1994). Songer et al observed that nondiabetic siblings (who are at high risk of the disease) were closer in height to their diabetic siblings than were the low risk sibs (Songer et al. 1986).

In twin studies (Leslie et al. 1991), the growth of the twin who subsequently developed diabetes was slower 1-2 years before diagnosis compared to the co-

twin. However, the studies of Blom et al, Price and Burden and the large epidemiological study of Hypponen clearly showed more rapid growth in those children who subsequently developed diabetes (Blom et al. 1992; Price and Burden 1992; Hypponen et al. 2000).

1.4.2.2 Growth after Diagnosis

Although DuCaju (Du Caju et al. 1995) reported normal growth in a longitudinal study of Belgium children with T1D, most studies have detected a loss of height SDS in the years following diagnosis (Lee et al. 1984; Thon et al. 1992; Brown et al. 1994; Holl et al. 1994; Tillmann et al. 1996; Salerno et al. 1997; Ahmed et al. 1998; Bognetti et al. 1998; Holl et al. 1998a; Huang et al. 2001). In a retrospective study, Donaghue (Donaghue et al. 2003) compared the height SD scores of two groups of T1D children. One group was diagnosed between 1974 and 1990 and the second group between 1991 and 1995. Summarising their data in the following table:

Ht SDS (mean (sd))	Diagnosed 1974-90	Diagnosed 1991-95
At diagnosis	0.28 (1.01)	0.38 (0.99)
5 years follow-up	0.07 (0.99)	0.37 (0.94)
10 years follow-up	0.04 (0.87)	0.36 (0.89)

Table 1.4a, T1D Children: Height SDS from diagnosis, Donaghue

It can be seen that the more recently diagnosed children have not had a loss of height SD scores from diagnosis. The authors ascribed this improvement in growth to intensification of insulin therapy that had occurred over the years.

1.4.2.3 Pubertal growth

Some studies have suggested that the timing of peak height velocity may be delayed in children with diabetes (Du Caju et al. 1995; Tillmann et al. 1996) but the longitudinal study of contemporary children with T1D has not confirmed this

(Ahmed et al. 1998) nor have the studies of Salardi, Brown or Donaghue (Salardi et al. 1987; Brown et al. 1994; Donaghue et al. 2003). There appears to be general agreement that the pubertal growth spurt in subjects with T1D may be blunted, particularly in girls (Salardi et al. 1987; Brown et al. 1994; Du Caju et al. 1995; Jos et al. 1996). In a few of the early studies (Jivani and Rayner 1973; Lee et al. 1984) the timing of PHV was reported to be delayed, but later studies report normal timing. The Oxford study of Brown et al (Brown et al. 1994) indicated that those who were diagnosed under the age of 5 years experienced the greatest pubertal height loss. Salardi et al (Salardi et al. 1987) reported that girls diagnosed just before puberty had the poorest pubertal growth.

1.4.2.4 Final height

Although there is a wide reporting of loss of height SDS from diagnosis, the general consensus is that most contemporary children with T1D will obtain a final height that is in the normal population range and appropriate for their family (Zachrisson et al. 1997; Scheffer-Marinus et al. 1999; Huang et al. 2001). The study by Luna et al showed that in addition to the ht SDS loss from diagnosis, the boys but not the girls had a final height SDS of –1.02 (Luna et al. 2004). It may be that there is an underestimation of final height in many studies as they end before final height is achieved. Heinze et al (Heinze et al. 1993) observed that growth might continue (albeit slowly) in diabetic subjects until the age of 20 years.

In a retrospective analysis of final heights on 181 subjects with T1D, Penfold et al (Penfold et al. 1995) observed a reduced final height (ht SDS=-0.22) that was also low relative to the subject's family. However, other studies have found heights very near the mean for the population: Brown et al (Brown et al. 1994)

on 80 children followed to final height observed a final height SDS of –0.06, Holl et al (Holl et al. 1994) reported normal final heights on 76 of their subjects, Huang (Huang et al. 2001) reported final height SDS's for girls = -0.05 and for boys = -0.13. Thus it may be that conclusive data are still to come and that our current knowledge is compounded by comparisons of different populations with varying ages of diagnoses, glycaemic control and insulin therapy.

1.4.2.5 Growth in relation to HbA1c

Although it is tempting to speculate that poor glycaemic control will lead to poor growth, it has been difficult to prove a relation between growth and glycaemic control as measured by HbA1c. Numerous studies have been unable to detect any relationship between HbA1c and growth in children with T1D (Jivani and Rayner 1973; Hjelt et al. 1983; Clarson et al. 1985; Salardi et al. 1987; Herber and Dunsmore 1988; Thon et al. 1992; Pitukcheewanont et al. 1995). The retrospective cross-sectional design of many of these studies may have contributed to these discrepancies. Three longitudinal studies identified a relationship between growth and HbA1c: Wise et al (Wise et al. 1992) reported a significant relationship between the two that was more evident in the prepubertal years; Gunczler et al (Gunczler et al. 1996) in a group of 79 subjects followed for 5 years from diagnosis, showed that children with poor control (HbA1c>9%) had a significantly lower growth velocity over the five year period than children with better control (lower HbA1c); and in their prospective study, Ahmed et al. (Ahmed et al. 1998) demonstrated a negative relationship between peak height velocity SDS and HbA1c in both sexes. Holl et al (Holl et al. 1998a) demonstrated a significant reduction in final height in children with poor control compared to those with good control.

1.4.2.6 Growth and insulin dose

In spite of the importance of insulin in the regulation of IGF-I and the importance of both in normal growth, peripheral insulin delivery does not correct the imbalances that exist; only portal administration of insulin will totally correct these abnormalities. Showing an improvement or relationship in growth relative to changes in insulin therapy has not been easily achievable. Ahmed et al (Ahmed et al. 1998) found no relationship between insulin dose and PHV SDS in their longitudinal study. One study that has shown an improvement in growth due to intensified insulin therapy has been that of Rudolf et al (Rudolf et al. 1982). Donaghue et al (Donaghue et al. 2003) analysed 2 cohorts separated in time and demonstrated that the more recently diagnosed group with more intensive therapy did not lose height SDS 5 and 10 years from diagnosis.

1.4.3 Timing of Puberty

Some of the studies over the past 20 years have reported conflicting observations regarding the timing of pubertal onset; DuCaju et al (Du Caju et al. 1995) reported a noticeable delay in boys and Tillman (Tillmann et al. 1996) and Salardi (Salardi et al. 1987) in girls while Clarson et al (Clarson et al. 1985) found no delay in their subjects of either sex. Codner et al (Codner et al. 2004) in their cross sectional study of 100 Chilean girls with T1D found no difference in the onset of puberty between the T1D girls and controls but did observe a later age of menarche and mean age of breast stages 3, 4 and 5. Summarising this in the following table from their data:

	T1D		Controls	
	Mean Age	N	Mean Age	N
B2	9.1	23	8.9	103
B3	11.1	17	10.4	90
B4	12.2	15	11.7	72
Menarche	13	100	12.5	576
B5	13.3	30	12.8	167

Table 1.4b, Girls: T1D vs Controls; Ages at reaching different puberty stages and menarche, Codner

The Oxford study of Ahmed et al (Ahmed et al. 1998) showed the age of menarche for girls with T1D was not significantly different from the mean population age. Summarising from this data, ages for menarche, PHV and the onset of puberty for both sexes are given in the following table:

Ages	Sex	T1D	Controls
Onset of puberty	Boys	12.1 ± 0.7	11.6 ± 1.1
	Girls	10.9 ± 0.9	11.2 ± 1.1
PHV	Boys	14.0 ± 1.3	14.1 ± 0.9
	Girls	12.0 ± 0.7	12.1 ± 0.9
Menarche	Girls	13.2 ± 0.7	13.2 ± 1.0

Table 1.4c, Girls and Boys: T1D vs Controls; Age of pubertal onset, PHV and menarche, Ahmed

The Australian study of Donaghue (Donaghue et al. 2003) showed no evidence of pubertal delay; there were no differences between the children with T1D and controls. In addition, there was no significant secular trend.

1.4.4 Body Composition

There is a tendency for weight or weight for height to be greater in children with T1D compared to controls in both sexes (Thon et al. 1992; Holl et al. 1994; Pitukcheewanont et al. 1995; Holl et al. 1998b) or only in girls (Mortensen et al. 1988; Gregory et al. 1992; Bognetti et al. 1995; Du Caju et al. 1995; Danne et al. 1997).

Most of these studies were cross sectional and used weight or BMI as an estimate of fatness (with the exception of Gregory et al); instead of any measure

of body composition. Another cross sectional study by Codner et al (Codner et al. 2004) on 100 girls with T1D (divided into the 5 pubertal stages) observed that BMISDS increased throughout puberty while it remained relatively stable in the control girls. The waist-hip ratio decreased during puberty in the control girls but not in the girls with T1D.

In the prospective longitudinal body composition study of Ahmed et al (Ahmed et al. 2001), an increase of BMI was seen in all children as they progressed through puberty. When the analysis was done by body composition, it was observed that the girls increased their body fat with puberty while the boys had a decrease in percent body fat. Comparing these gains to a control group, although both groups of girls gained fat mass, the T1D girls gained significantly more fat mass than controls. Both control and T1D boys lost percent body fat but the loss was greater in the T1D boys than in the control group.

There is general consensus that excessive weight for height might relate to insulin dose or frequency of insulin injections in subjects with T1D (Anonymous 1988; Reichard et al. 1991; Gregory et al. 1992; Anonymous 1995; Bognetti et al. 1995; Danne et al. 1997; Mortensen and Hougaard 1997; Holl et al. 1998b).

1.4.5 Endocrinology of Puberty and Growth in T1D

1.4.5.1 Adrenal androgens

There are only a small number of published studies on adrenal androgen levels in T1D and these have reported conflicting results. Several groups have observed levels of DHEAS that are similar in their T1D subjects and controls: Meyer et al (Meyer et al. 2000) in a cross sectional study of T1D pubertal subjects (36 boys and 31 girls) and 59 controls found that levels of DHEAS were similar between subjects and controls. In an earlier study, Small et al (Small et al. 1989) reported comparable results in a group of 17 young adult males with

T1D and age matched controls for both DHEAS and androstenedione and commented that they also had observed similar results in females.
Other groups, however, have found lower levels of DHEAS in their T1D subjects; Cohen et al were the first to look at serum DHEAS levels in subjects with type I diabetes and they observed lower levels in their subjects than controls (Cohen et al. 1984). Loviselli et al (Loviselli et al. 1994) also found low levels of DHEAS in a cross sectional study of 15 young males with T1D compared to 18 controls. In a study of 129 children (12.6 ± 3.8y) with T1D and 458 age-matched controls, Radetti et al (Radetti et al. 1994) reported a DHEAS-SDS for the T1D children of -0.36 ± 0.77 and this was significantly different from zero.
The mechanism of how insulin might affect DHEAS is not clear. It has been shown that experimentally induced hyperinsulinaemia decreases adrenal androgen levels in humans by inhibiting the 17, 20 lyase activity (Nestler et al. 1992). Ebeling (Ebeling et al. 1995) gave an acute infusion of insulin in 23 young adult males with T1D that lowered the levels of DHEAS by 11% ($p<0.001$). In an earlier study, Nestler (Nestler et al. 1989) had conjectured that supraphysiologic levels of insulin might act either via the insulin receptor or IGF-I receptor.

1.4.5.2 GH

Studies on the GH/IGF-I axis in T1D has been a topic of research for at least the last 20 years (Amiel et al. 1984; Bloch et al. 1987; Fowelin et al. 1991). It is well known that spontaneous overnight growth hormone secretion is higher during puberty in subjects with T1D compared to controls (Edge et al. 1990; Clayton et al. 1994). The highest levels in both groups were noted in puberty stage 2/3 in girls and 4/5 in boys corresponding to the time of peak height velocity. The

mean overall GH levels are increased at all puberty stages with increases in both pulse amplitude as well as baseline concentrations. Halldin et al (Halldin et al. 1998) have studied daytime levels of GH in a group of late pubertal girls with T1D. They observed that daytime levels were also elevated. This excessive GH secretion results in an increase in insulin resistance (Press et al. 1984), which may only exacerbate that seen during normal puberty. Although in normal children it has been suggested that the hyperinsulinaemia of puberty may enhance growth, the diabetogenic effects of elevated GH levels in T1D subjects (with subsequent antagonistic effects on insulin) may decrease the optimal growth potential of these children.

1.4.5.3 IGF-I

Paradoxically the levels of insulin-like growth factor–I (IGF-I) in subjects with T1D are lower than in puberty-matched controls (Amiel et al. 1984; Taylor et al. 1988) as are that of its carrier protein IGFBP3 (Batch et al. 1991). This would appear to be analogous to a GH resistant state; ie high levels of GH that do not produce increased levels of IGF-I and these low levels then feedback to encourage a continuing increase in GH production.

It is known that in the portal hepatic circulation of T1D subjects, insulin levels are low as are levels of IGF-I, this results in higher levels of the low molecular binding protein IGFBP-1. This binding protein has been found to exert an inhibitory influence on IGF-I (Taylor et al. 1990) and its high levels may reduce the bioavailability of IGF-I (and therefore add to the high levels of GH observed). The low levels of IGF-I in T1D have been cited as a possible cause of the observed diminished pubertal growth. Although the growth spurt has been seen to be sub-optimal in girls more often than boys, it is the IGF-I levels in boys that have been reported to be lower than in girls (Ahmed et al. 1998). Thus this can

only be part of the answer for the diminished pubertal growth, and it may be that growth hormone acts directly on the growth plate or that the effects of the sex steroids on the GH/IGF-I axis play more of a role in pubertal growth than IGF-I itself.

1.4.5.4 Insulin

In T1D, levels of insulin are high in the peripheral circulation and low in the portal vein. It has been demonstrated that it is these portal vein levels that are important to maintain normal levels of IGF-I (Dunger et al. 1993). Insulin delivery into the portal circulation is not clinically possible although it has been shown to normalise the derangements of T1D (Shishko et al. 1992).

Although IGF-I is regulated by GH, insulin enhances IGF-I secretion by its effect on the hepatic growth hormone receptor or by a permissive effect on post receptor events (Baxter and Turtle 1978; Maes et al. 1986).

In T1D, the insulin resistance of puberty cannot be compensated for by increased insulin secretion and so to help counter this, the insulin requirements of subjects with T1D increase substantially during puberty from a usual 0.25-0.5 units/kg/day to 1.0-1.5 units/kg/day. This usually diminishes after the attainment of PHV and levels gradually decrease to prepubertal values. However, the intensification of insulin therapy that is generally necessary to maintain reasonable glycaemic control has often meant that unacceptable weight gain has ensued especially in the girls during late puberty (Anonymous 1988; Ahmed et al. 2001). In a longitudinal study, we have shown that young girls with T1D have a significant greater amount of body fat compared to controls in late adolescence (Ahmed et al. 2001). Since insulin is regarded as both a hormone that can potentiate growth (Caprio 1999) and possibly puberty (through its effect on SHBG (Holly et al. 1989)), it is perhaps not surprising that children with T1D

who are reasonably well controlled on adequate insulin replacement will, in general, grow and develop normally.

1.4.5.5 SHBG

SHBG is important for the transport of the sex hormones in the circulation and instrumental in determining their bioavailability (Rosner 1990). In normal subjects it was seen that SHBG decreased during puberty and that insulin and IGF-I (in boys) correlated negatively to SHBG (Holly et al. 1989). Since it is known that levels of IGF-I and portal insulin levels are low during puberty in children with T1D and in vitro work has shown insulin to decrease SHBG from a human hepatoma cell line (Singh et al. 1990; Loukovaara et al. 1995; Kalme et al. 2003), it was thought that the levels of SHBG might be high in T1D children during puberty. The cross sectional study in T1D adolescent subjects of Holly et al did not observe raised SHBG levels compared to controls (Holly et al. 1992), it did, however, show a decrease of SHBG with advancing puberty stages. This is in contrast to a study by Christensen et al on adult men with T1D where an increase in SHBG compared to controls was observed (Christensen et al. 1997). The study of Meyer et al on 67 (36 boys) pubertal subjects with T1D found no difference in the levels of SHBG at any stage of puberty between the T1D group and normal controls (Meyer et al. 2000). Whereas Rudberg and Persson observed lower absolute SHBG levels in young women (puberty stage 4-5) with T1D compared to controls as well as an inverse relationship of insulin dose and SHBG (Rudberg and Persson 1995). In Holly's study, the prepubertal T1D children had lower levels of SHBG compared to controls and showed no sex difference. It was also observed that neither the duration of diabetes nor metabolic control (HbA1c) related to SHBG but there was a weak correlation to insulin dose however, observed only in the pubertal boys (Holly et al. 1992).

Although not reported these authors say they found no abnormalities of sex steroids in these subjects.

1.4.5.6 Leptin

The discovery of the hormone leptin (Zhang et al. 1994) which is produced by adipocytes and regulates appetite, food intake and energy metabolism in rodents (Maffei et al. 1995; Vaisse et al. 1996) and humans (Montague et al. 1997) might help to explain the often reported gains of excess weight in subjects with type 1 diabetes (Gregory et al. 1992; Thon et al. 1992; Holl et al. 1994; Bognetti et al. 1995; Du Caju et al. 1995; Pitukcheewanont et al. 1995; Danne et al. 1997; Holl et al. 1998b).

The mechanism of excessive weight gain for height in pubertal subjects with T1D has not been fully explained. A relationship to insulin dose or frequency of insulin injections has often been cited, particularly in girls (Anonymous 1988; Anonymous 1995; Danne et al. 1997; Mortensen and Hougaard 1997; Holl et al. 1998b). Although there remain some discrepancies in the literature (see below) most reports are of raised leptin levels in children and adolescents with T1D compared to control children (Kamoda et al. 1998; Kiess et al. 1998; Luna et al. 1999; Bideci et al. 2002; Soliman et al. 2002; Morales et al. 2004). The longitudinal study of Ahmed et al showed that increased levels of leptin existed in both the T1D boys and girls but it was only the girls who gained more body fat than controls (Ahmed et al. 2001).

It has been postulated that this might reflect 'leptin resistance' resulting from the peripheral hyperinsulinaemia that invariably occurs in T1D treated with subcutaneous rather than portal insulin administration (Dunger 1992). Not all studies, however, have reported elevated leptin in T1D children, Verrotti et al, Myers et al and Karaguzel et al did not find any difference in leptin levels

between children and young adults with type 1 diabetes and matched controls (Verrotti et al. 1998; Myers et al. 2004; Karaguzel et al. 2006); whereas Kirel et al (Kirel et al. 2000) found lower leptin levels in their diabetic children compared to controls.

It is interesting to note the 'permissive' quality of leptin with regards to puberty (see section 1.3.8). It has not been reported that children with T1D go into puberty at earlier ages than controls which one might expect with higher levels and if leptin was the 'trigger'.

1.4.5.7 Sex Steroids

1.4.5.7.1 Testosterone

In one of the earliest studies looking at testosterone in T1D women, Djursing saw slightly elevated SHBG and significantly high testosterone in T1D women with peripheral hyperinsulinaemia (Djursing et al. 1985). A few years later, Small observed testosterone levels in 17 postpubertal T1D subjects; all were in the normal range and although slightly higher levels of testosterone were observed in the T1D subjects, there was no significant difference (Small et al. 1989). Rudberg and Persson (Rudberg and Persson 1995) in a small cross sectional study of 25 T1D young women in late puberty (stage 4-5) observed low SHBG and higher testosterone levels in T1D than control subjects. Meyer et al (Meyer et al. 2000) reported conflicting results from a cross sectional study of 67 subjects. They observed significantly higher total testosterone and free testosterone in both females ($p<0.05$) and males ($p<0.01$) with T1D than in controls at puberty stage 5 but not in any earlier stages. Salardi et al (Salardi et al. 2002) also found higher testosterone levels in T1D stage 5 male adolescents and young adults (14.2-33.3y) with a median duration of diabetes of 12 years. In one recent study of 50 men (28-51y), Tomar (Tomar et al. 2006) found that

more than 90% of patients had a normal testosterone and free testosterone and this agreed with Grossman (Grossmann et al. 2008) who studied 69 men (mean age 45y) with T1D and found 7% had low (<10nmol/L) total testosterone (equal percent to controls) and 20% had low calculated free testosterone (greater percent than controls). In recent years, the work of Codner and Escobar-Morreale on PCOS in T1D women has focused on the consistent observations of hyperandrogenism in this group of patients (Escobar-Morreale et al. 2000; Codner and Escobar-Morreale 2007).

1.4.5.7.2 Oestrogen

There is little easily found published work on oestrogens in T1D children or adolescents. Most of the work in T1D women has been on post menarcheal adolescents or women comparing menstrual cycle regularity to controls or comparing amenorrhoeic and non-amenorrhoeic diabetic women. Early work by Ciognani et al (Cicognani et al. 1978) claimed normal gonadotrophins but reduced responses to LHRH in 14 diabetic boys and concluded a limited capacity to maintain an adequate pituitary reserve. A disorder of the hypothalamus/pituitary/ovarian axis was suggested (Schriock et al. 1984) following the observation that girls diagnosed after the age of 11 had a delayed menarche compared to controls. This was confirmed by the studies of both (Burkart et al. 1989) and (Kjaer et al. 1992) who found that the age of diagnosis was inversely related to the age of menarche. None of this, however, specifically tells us about E2 levels in puberty. In a discussion by Danielson et al (Danielson et al. 2005) on the effect of HbA1c on menarcheal age in T1D women, they mention three references with conflicting results: (Djursing et al. 1982), decreased E2 levels in T1D;(Zumoff et al. 1990) increased levels and (Djursing et al. 1985) similar levels to controls. In a recent study of ovarian

function in T1D girls, Codner et al observed little difference in basal oestradiol levels in T1D girls and puberty matched controls (Codner et al. 2005). Thus the paucity of available information does not really allow a clear picture on oestradiol in T1D pubertal children compared to a control group.

1.4.5.8 Thyroxine

Since normal growth and pubertal development is dependent on normal thyroid levels and children with T1D have an increased incidence of autoimmune thyroid disease, it is imperative to determine the thyroid status of youngsters with T1D. In the 1980's a number of reports published conflicting results on thyroid hormone concentrations in children with T1D with some saying levels were no different to control children (Gilani et al. 1984; Sluszkiewicz 1986) while others reported lower levels in T1D compared to controls (Salardi et al. 1984; Trimarchi et al. 1984; Dorchy et al. 1985; Chiarelli et al. 1989). Bernasconi et al reported that total hormone levels T3 and T4 were lower as was TBG but the free T4 and T3 were normal (Bernasconi et al. 1984). A more recent longitudinal study by Connors and Dunger also reported low levels of total T4 and TBG but found freeT4 within normal limits for both sexes (Connors et al. 1996). These authors also observed that although duration of diabetes had no effect on thyroid hormone concentrations, there was a negative association between total T4 or freeT4 and HbA1c. This latter was in general agreement with other reports although Trimarchi et al observed a negative correlation between disease duration and thyroid hormone levels (Trimarchi et al. 1984).

1.5 Appraisal of previous research

- The relationships between hormones, glycaemic control and growth have proved difficult to define in T1D children. The often small and cross-

sectional or mixed cross-sectional design of many studies of pubertal growth in normal children as well as those with T1D may contribute to the lack of consensus. To explore events during puberty and reflect changes over time, a longitudinal design is essential.

- There have often not been contemporary longitudinal controls seen at visits frequent enough to examine pubertal events.
- There appear to be no published longitudinal studies in normal children or children with T1D that compare hormones, growth and pubertal events throughout the whole pubertal period.

1.6 Questions to be addressed by the current study:

The aim of this research was to explore elements of pubertal growth and development and body composition changes in a group of children without diabetes and to do the same in children with diabetes including measures of HbA1c levels and insulin dose. In particular, to explore whether it was possible to clarify relationships between these variables and hormones with pubertal growth and determine what if any association they might have on the age of pubertal onset, PHV and height SDS change both within and between the two cohorts. The cohorts comprised 52 T1D children (27 boys, 25 girls) aged 7.7-14.4yrs and 125 control children (54 boys, 71 girls) aged 8.3 -11.96 yrs at first visit followed until 16.6 years of age. My objectives were:

1. To examine within each cohort some components of pubertal growth including age at onset of puberty, peak height velocity, age of menarche and a measure of pubertal duration.
2. To explore within each cohort aspects of body composition (fat and fat-free mass) changes during puberty.

3. Comparison of the above in the two cohorts.
4. Hormonal comparisons during puberty between the two cohorts.
5. To explore possible relationships between various growth and pubertal variables and hormones.

In summary the main goals of this study are to explore the differences in pubertal growth between children with and without type 1 diabetes with special emphasis on the hormonal, pubertal and body composition changes that are occurring at this time.

Chapter 2. Subjects and Methods

2.1 Subjects: Description of the two cohorts

2.1.1 Controls

The control group comprised normal healthy children from a comprehensive school in Chard, Somerset, who had participated in a prospective longitudinal study of normal growth and hormonal changes during puberty in the 1980's. This study was conceived and directed by Professors Preece and Dunger who have kindly allowed me to use the data.

The children and their parents were seen in the four feeder primary schools to introduce the study before the children went into the upper school. This was to ensure an entry age, particularly in the girls, early enough to ascertain the start of puberty. Ethical approval was obtained from the Hospital for Sick Children, Great Ormond Street and written consent was obtained from the parents with the assent of the children. The children were seen six monthly when measurements of height, weight, skinfolds and pubertal assessments were made. All measurements were done by one of three observers and all the girls' puberty assessments were made by one female observer while one of two male investigators assessed the boys. All observers originally trained in the Department of Growth and Development at the Institute of Child Health, London. Blood samples were taken at each visit for IGF-I, leptin, testosterone, oestradiol, androstenedione, dehydroepiandrosterone sulfate (DHEAS), sex hormone binding globulin (SHBG), and thyroid function. There were originally 71 girls and 54 boys enrolled in the study; however, owing to attrition and lack of data due to a variety of reasons (Professor Preece, personal communication), there are data on 39 girls with peak height velocity, 47 with reliable menarche ages,

and 24 boys with peak height velocity. No data on final height has been examined as the children were only followed until 16 years of age. Longitudinal hormonal data from 6 monthly measurements exists for varying numbers of subjects from 18 to 27.

Table 2.1a has details of the children's ages and number of visits:

	GIRLS		BOYS	
	Median	Range	Median	Range
At first visit	9.6	8.3-11.96	9.48	8.3-10.2
At last visit	15.8	10.1-16.6	15.6	9.5-16.5
Time in study	6.42	0.5-6.43	6.42	0.5-6.42
No of visits	13	1-14	12	1-14

Table 2.1a: Ages at first and last visit, time in study, number of visits (all; median and range in years)

This 'Chard' cohort has been used as the contemporary control group for the current study of T1D subjects to compare age at pubertal onset, age and magnitude of peak height velocity, pubertal maturation, hormonal differences during puberty and to explore the hormonal effects on growth and puberty. The references of Tanner et al have been used to assess the representativeness of this sample in terms of pubertal onset, tempo of growth and ages of menarche and PHV in particular, the data from the Marshall and Tanner puberty papers has been used to compare ages at puberty stages and menarche (Marshall and Tanner 1969; Marshall and Tanner 1970). The Freeman et al reference was used to calculate height and BMI standard deviation scores (Freeman et al. 1995).

2.1.1 T1D cohort

The study of the Oxford children with diabetes was modelled on the Chard study incorporating measurements of growth and puberty assessments as well as blood sampling for hormonal determinations.

Fifty-two prepubertal children (27 boys, 25 girls) with type I diabetes mellitus (T1D) attending the paediatric diabetes clinic at the John Radcliffe Hospital in Oxford were recruited over 8 years for a longitudinal study in growth and puberty in the late 1980's. Sixteen girls and nineteen boys were followed to final height (defined as a height velocity <1cm/y). Further subject details are in Table 2.1b:

	GIRLS		BOYS	
	Median	Range	Median	Range
At first visit	9.9	7.7-12.5	11.15	10-14.4
At last visit	15.8	10.8-18.1	17.96	12.8-19.8
Duration of diabetes at first visit	2.4	0.64-9.15	6.57	0.9-11.9
Time in study	5.9	1.04-8.36	6.3	2.1-8.9
Age at diagnosis	7.48	1.5-11.3	4.28	1.1-10.64
No of visits	19	4-26	21	8-34

Table 2.1b: Ages at first and last visit, duration of diabetes, time in study, age at diagnosis and number of visits (all; median and range in years)

The hospital is a regional specialist centre in childhood diabetes and receives referrals from throughout the district. Oxfordshire is a diverse area socially and economically; it has a mixture of rural and light industries and is set amongst farmland and small market towns with some larger conurbations.

The Somerset area where the control children came from was comparable to the Oxford region with a socio-economic mix that was similar. All the children in both groups were from British/European backgrounds.

As each child in clinic approached the age when it might be feasible to think puberty was imminent, in discussion with the consultant and then the child and family, MLA explained that their growth and puberty status would be carefully monitored at each clinic visit and asked if they were agreeable to this. All participants and their parents gave verbal agreement. This approach was regarded as part of their routine clinical surveillance as well as trying to gain an

insight into the hormonal issues around puberty relating to growth. As such this study came under the umbrella of the ethics granted to a more invasive study at the time exploring overnight hormonal variation in adolescent patients with T1D. Only one girl withdrew after a few visits since she did not wish to have her pubertal status assessed, three other children moved out of the area after several visits and one child suffered DKA and cerebral oedema during the course of the study and so further assessments on him ceased.

The children were seen three or four times a year as part of their normal clinic routine when height, weight, skinfold measurements and Tanner pubertal staging was assessed. MLA took all the measurements on all the children and assessed the girls' puberty status; various male clinicians assessed the boys' puberty. (All the observers involved in the Chard study as well as MLA were originally trained in auxological techniques in the department of Growth and Development at the Institute of Child Health, London in the 1970's. Although no inter-observer reliability was possible, they were all experienced and highly trained.)

Blood samples were taken annually for C peptide; six monthly for IGF-I, leptin, testosterone, oestradiol, androstenedione, dehydroepiandrosterone sulfate (DHEAS), sex hormone binding globulin (SHBG) and thyroid function; and at each visit for HbA_{1c}. MLA made annual bone age assessments using the Tanner-Whitehouse 2 RUS system (Tanner et al. 1983).

At recruitment, all of the T1D children were on two injections of intermediate and soluble insulin daily, but during puberty 75% were changed to multiple injection therapy with three pre-prandial injections of soluble insulin with an intermediate acting insulin given last thing at night before bedtime.

2.2 Methods

2.2.1 Anthropometry

All measurements were taken using standard auxological techniques as described by N Cameron (Cameron 1984). A full description follows.

Height, Weight and Skinfold measurements

Height was measured at each visit using a Harpenden stadiometer (Holtain Ltd., Crosswell, Crymmych, Dyfed, Wales); this was a portable one in the case of the control subjects. The subjects removed their shoes and stood with their backs against the backboard with the back of their heels, buttocks, scapulae and head touching the board while they looked straight ahead. The head is in the Frankfurt Plane (so that a line drawn from the lower orbit of the eye to the external auditory meatus is parallel to the floor), the subject is asked to take a breath in and then relax while gentle traction is applied to the mastoid processes. The measurement is read to the nearest completed millimetre on expiration.

Weight was measured to the nearest 0.1kg using a Marsden digital scale (Henley on Thames, Oxford) for the T1D subjects and a portable beam balance for the control subjects. All were weighed in normal indoor clothes with shoes removed.

Subcutaneous skinfold measurements were taken at four sites: biceps, triceps, subscapula and suprailiac with a Holtain skinfold caliper (Holtain Ltd) on the left side of the body. The location for the measurement of the biceps and triceps skinfold was at the half-way point between the acromion and the olecranon when the arm was bent at a right angle and this position was marked with ink on the lateral aspect of the subject's arm. All the skinfolds are made with a

sweeping motion of the forefinger/middle finger and the thumb of the observer's left hand. The dial in all cases is read to the nearest completed 0.1mm.

Biceps: The observer faces the subject and picks up the skinfold on the anterior aspect of the arm with the forefinger/middle finger and thumb of the left hand just above the mark where the caliper will be applied, while maintaining this 'pinch', the observer applies the caliper with the right hand.

Triceps: The subject stands with his back to the observer while the observer picks up the skinfold on the vertical axis of the upper arm midway between the lateral and medial surfaces of the arm. Again the observer grips the fold with the forefinger and thumb of the left hand slightly higher than the ink mark and applies the caliper at the level of the mark while continuing to maintain the pinch.

Subscapula: The observer stands behind the subject who is asked to stand in a relaxed position. Using the forefinger and thumb of the left hand the observer 'sweeps' along the angle of the scapula to the point of the inferior angle where the skinfold is picked up.

Supra-Iliac: The subject faces the observer who picks up the skinfold at a point that is 1cm above and 2cm medial to the anterior superior iliac spine (ASIS). This is palpated with the thumb of the right hand while holding the caliper and the vertical pinch is then applied with the left hand above the point where the caliper is then applied.

Reliability: This observer has carried out test-retest studies and performed an analysis of the differences for both height and the four skinfold measurements. Although there are numerous and often a confusing array of reliability statistics in the literature, the technical error of the measurement (TEM) has been used here to express the precision of the measuring. The interpretation is that 95%

of the observer's measurements will be within ± 1.96 of her/his TEM. The calculation of TEM = $\sqrt{}$ ($\sum$differences^2/2N). A summary of the results is presented here and the raw data are in the Appendix, section 8.6.

	Height, cm	Biceps, mm	Triceps, mm	Subscapula, mm	Suprailiac, mm
N of pairs	34	36	36	36	36
$\sum$ diffs^2	1.37	11.98	17.98	7.26	8.09
$\sum$ diffs^2/2N	.02	.17	.25	.10	.11
TEM	.14	.41	.50	.32	.34
TEM*1.96	.28	.80	.98	.62	.66

Table 2.2a, TEM results for this investigator

These values compare well with those quoted by Van den Broeck (van den Broeck 2001) (www.growthanalyser.org) who states that an experienced observer should have a TEM for height <0.25cm and for triceps skinfold measurement <0.55mm and subscapular skinfold measurement<0.45mm. Ulijaszek and Kerr (Ulijaszek and Kerr 1999) have summarised 29 papers reporting reliability statistics from 1972 to 1997 and again the values here agree well with their review (see table below).

More recently, a WHO study group (WHO 2006) reported the reliability in their international multicentre growth centres and published both their expert's TEMs and the range for the team of measurers and these are presented in the far two columns of the table.

	No of studies	TEM mean	TEM range	WHO TEM expert	WHO TEM range of teams
Height	19	0.38	0.1-1.3	0.23	0.16-0.29
Biceps	3	0.17	0.1-0.2		
Triceps	21	0.84	0.1-3.7	0.40	0.39-0.61
Subscapula	19	1.26	0.1-7.4	0.30	0.29-0.41
Suprailiac	10	1.16	0.1-3.2		

Table 2.2b, TEM results for WHO investigators

2.2.2 Bone Ages

A left hand and wrist x-ray was taken annually on the T1D subjects according to the specifications in the manual (Tanner et al. 1983). Bone ages were assessed using the 13-bone system that assesses the epiphyses of the radius, ulna and short bones (metacarpals and phalanges) (RUS) of the first, third and fifth ray. Each bone is considered individually and assigned a stage that is defined by up to three criteria (8 stages (a-h) for the ulna and 9 (a-i) for the radius and short bones). An individual score is assigned to each and these are summed to a total skeletal maturity score. This score is then converted to the TW2RUS bone age using a population specific centile chart.

No formal assessment was made at this time for MLA's bone age reliability. However, she was trained by one of the originators of the system (RHW) and had in the past performed reliability estimates and the paper by Beunen and Cameron (Beunen and Cameron 1980) demonstrated such good reproducibility for the TW2RUS system that no further study by MLA was performed.

2.2.3 Hormone Measurements

Most of the measurements were made in the same laboratories at the same time for the two studies. Jen Jones at the Institute of Child Health kindly performed all the IGF-I estimations. The adrenal androgens and SHBG were done in Les Perry's laboratory at St Bartholomew's Hospital, London and Dave Morrell at the University of Edinburgh did the leptin assays. Thyroid hormone and sex steroids were done in the biochemistry dept at the John Radcliffe Hospital, Oxford for the T1D samples and in Dr Les Perry's lab at St Bartholomew's Hospital, London for the control samples. Hormone assays over the study period of 12 years changed from predominantly in house assays to

either commercial kits or to (semi) automated endocrine analysers. In all instances before being used the new methods were validated and demonstrated to be comparable to the previously used methods. Details of methods with coefficients of variation are given in the Appendix, section 8.9.

2.2.4 Analytical Methods

2.2.4.1 PHV

2.2.4.1.1 Description of graphical technique

Peak height velocity (PHV) was determined by plotting each child's height data on an exaggerated scale (x2) and a 'best fit' line was drawn by eye with the aid of a flexible lead spline. The height values were then read off every 0.25-year (3 monthly) from this smoothed distance curve and annual velocities were calculated and plotted at the midpoint of each whole year for every 0.25 of a year. These values were plotted and a smoothed curve was then drawn through these points. Both the age and magnitude of PHV was read from this smoothed velocity curve.

2.2.4.1.2 Comparison to Preece-Baines

In their original 1978 paper, Preece and Baines compared their model's assessment of PHV magnitude and age of PHV to that obtained by the graphical smoothing technique (Preece and Baines 1978). Comparison for age of PHV was good with the graphical age being 13.83y for boys and 11.82y for girls corresponding to 13.77y and 11.80y respectively. There was, however, a significant difference for the magnitude of PHV; graphical 9.62cm/yr for boys and 8.32cm/yr for girls compared to 8.69cm/y and 7.58cm/y respectively. Graphical smoothing allows far more flexibility than mathematical modelling and can follow the data with no preconceived ideas. The user is not constrained by any preselected shape or parameters to be included. There are, of course,

drawbacks to graphical smoothing, it is laborious and time consuming, it relies on one observer and there is no expression of variability (no standard errors can be calculated). It was interesting to note that when a visitor in the department replotted and smoothed a number of the graphs that this investigator had previously done, that the two observers were able to agree incredibly closely on both the age and magnitude of PHV. See Appendix section 8.10.

With the kind help of Dr Mario Cortina Borja (Institute of Child Health, London) I have compared the graphical technique used in this study with a Preece-Baines age at PHV in both groups of subjects presented here. See Appendix, section 8.11.

2.2.4.2 SD Score calculations

Height and BMI SD Scores were calculated for each child for every visit using the Freeman et al references (Freeman et al. 1995). This was done using a program from the Child Growth Foundation (2 Mayfield Road, Chiswick, London W 4 1PW). It allows the user to enter the sex, age and variable of interest (i.e. height, weight, or BMI) of the child in an excel spreadsheet and calculates the SDS using the LMS method of Cole and Green (Cole and Green 1992).

An exception to this was the calculation of height SD scores for the diabetic cohort from diagnosis to final height these were done based on the Tanner et al references (Tanner et al. 1966a) as in the original paper (Ahmed et al. 1998).

Using the 1976 Tanner velocity charts for both sexes, the calculation of PHVSDS, adjusting for age of occurrence of PHV, was performed. Three parallel straight lines were drawn: one joining the 97th centile peaks for early, average and late spurting children, the second line joining the 50th centile peaks again for early, average and late children and the third for the 3rd centile for early average and late. For any age that a given child's PHV occurs, it is then

possible to read off from this graph both the mean PHV at that age and either the 3^{rd} or 97^{th} centile value. Using the relationship from the standard normal distribution of $\mu \pm 1.88\sigma = 3^{rd}$ or 97^{th} (and if we were to choose the 3^{rd} for example), solve for the $\sigma = (\mu - 3^{rd})/1.88$ for that age. Subsequently the formula $SDS = ((x - \mu)/\sigma)$ is utilised and the age adjusted PHVSDS for the child is obtained. (Where x = child's measurement, μ = population mean, σ = population standard deviation.)

2.2.4.3 Comparisons by PHV ± years

Once PHV age was ascertained in each child, this age was designated '0' and all measurements were aligned by ± years from PHV. The data was then grouped into yearly PHV age groups (± 0.5 year) centered on a whole year. Thus anyone seen from –0.50 to +0.49 years around '0' was grouped in the whole year '0' grouping. If a subject appeared in any group more than once, the data for that individual was averaged for that grouping.

2.2.4.4 Loess by SPSS

To explore hormonal changes during pubertal growth in relation to PHV, the loess method has been used to identify times of maximal hormone levels. SPSS uses a 'lowess' or 'loess' method to fit a smoothed curve to data. This is a method that is described as a 'locally weighted polynomial regression'. The acronyms 'lowess' or 'loess' are derived from the term 'locally weighted scatter plot smooth'. It is claimed that it combines the simplicity of linear least squares regression with the flexibility of non-linear regression. The process is regarded as local since each smoothed value is determined by neighbouring data points from within a defined range. It is weighted because a regression weight function is defined for the data points in the range. The local polynomials fit to each

subset of data are either locally linear or locally quadratic. The logic behind the method is that the points near the explanatory variable are more likely to be related to each other than points further away. Thus more weight is given to the nearest data points and less weight to those further away. It is claimed that this confers an advantage over some other methods in that it does not require a function to fit the model for all the data thus it is very flexible. (From Google online help)

2.2.4.5 Body Composition Equations

It is well accepted that the equations for the calculation of body composition are population specific, therefore this investigator chose those based on British children and adolescents (Durnin and Rahaman 1967; Brook 1971) even though there are more recent ones available. For the calculation of body density the following equations were used as appropriate:

Author	Age	Equation
Brook	1-11y boys	D = 1.1690 - 0.0788 x log (sum of 4 skinfolds)
	1-11y girls	D = 1.2063 – 0.0999 x log (sum of 4 skinfolds)
Durnin & Rahaman	12.7-15.7y boys	D = 1.1533 – 0.0643 x log (sum of 4 skinfolds)
	13.2-16.4y girls	D = 1.1369 – 0.0598 x log (sum of 4 skinfolds)
Siri		Percent body fat = ((4.95/density) – 4.5) x 100

Table 2.2c, Body composition equations

2.2.4.6 LMS/Cole

The distribution of the chosen measurement over a specific age range is summarised by the construction of three-smoothed age related curves using a cubic spline smoother (Cole and Green 1992). These 3 curves are: M, the median; S, the coefficient of variation of the measurement as it changes with age; and L the power transformation at each age that is needed so the data are

a Gaussian distribution. The three curves are combined to produce a set of centile or SDS curves.

The Dutch Growth Foundation (www.growthanalyser.org) has produced a database (Growth Analyser) using the Cole and Green method that allows a non-mathematical investigator (such as myself) to generate reference curves based on their own control data. The resulting LMS values can then be used to calculate SD scores for individual measurements for any group of subjects. This has been done in relation to years from PHV using the control children as the reference and plotting the T1D children on the resulting graphs (Appendix 8.12.2).

2.2.4.7 MLwiN

Multilevel modelling is an extension of ordinary multiple regression. It optimises the use of longitudinal data by not requiring an equal number of measuring occasions at equal intervals for each subject and thus uses all the available data (Baxter-Jones et al. 2003). In other analyses of repeated measures it has been necessary that the data conform to strict structures, that the children be seen at the same ages and the time intervals between visits be the same. In most longitudinal studies this is very difficult to achieve.

The development of this technique has allowed the handling of data where some children are seen only once (cross sectional) and others seen a number of different times at varying time intervals. It has allowed the exploration of relationships as well as construction of growth norms (Goldstein 1995). Its use on mixed longitudinal data permits the fitting of separate curves for groups (i.e. girls vs. boys or controls vs. study cohort) as well as individual growth curves (Round et al. 1999). Dr Ken Ong has kindly helped apply this technique on some of the body composition data reported here.

2.2.4.8 General Statistical Methods

In general, data are presented as means and sd unless otherwise expressed. All biochemical data were log transformed to approximate normal distributions and parametric analyses have been made. For non-normally distributed variables, nonparametric tests were performed. Differences between cohorts were by the 2 tailed Student's t-test on logged data or by the Mann Whitney U test on non-normal data. Pearson's correlation coefficients were used to explore relationships between hormone levels and growth/puberty parameters. Multiple linear regressions were employed to explore these relationships. Statistical significance was defined as $P \leq 0.05$. SPSS has been used (SPSS Inc, Chicago, Ill) the most recent being ver 10.

Chapter 3. Growth and Puberty in T1D and the Control Cohort

3.1 Control cohort and the Tanner references

The representativeness of the control children has been assessed by comparing them to the reference standards of Tanner (Tanner 1962; Tanner et al. 1966a) in terms of the age of pubertal onset, age of menarche, age of peak height velocity and tempo of growth.

3.1.1 Boys

3.1.1.1 Ages at Onset of Puberty and PHV

The control boys start puberty earlier compared to the UK references of Tanner, this is approximately equivalent to a difference of four and a half months. Comparison of PHVage between the two groups is, however, very similar. It may be that the onset of puberty in the Harpenden Growth Study boys (on which the Tanner standards are based) was delayed relative to a more representative population or it may be that the assessment of puberty via photographs (Harpenden) compared to 'in person' caused a difference or it may be related to the inherent variability in pubertal assessment itself.

	Controls	Tanner
G2	11.28 (0.78)	11.64 (1.07)
PHV age	13.82 (0.97)	14.06 (0.92)

Table 3.1a, Boys: Ages at Onset of Puberty and PHV, mean (sd)

3.1.1.2 Tempo of Growth

Comparing the control boys and the Tanner references (Table 3.1b) with regard to the distribution of genital stages at the time of occurrence of PHV demonstrates that in the control cohort, the distribution is spread more evenly over stages 3, 4 and 5 whereas three quarters of Tanner's reference group are

in stage 4 at PHV and nearly one quarter in stage 5. Again how much of this reflects a real difference or a difference in technique/subjectivity of pubertal assessments is difficult to assess. Combining stages 4 and 5 would give 71% in late puberty in our control group and 98% in the Tanner group. Thus most boys in both samples experience PHV in late puberty although 29% of our controls were in early puberty (stages 2-3) when PHV occurred indicating that our control boys were in all puberty stages at the time of PHV.

Genital stage		1	2	3	4	5
PHV	Controls	0	4	25	33	38
	Tanner	0	0	2	76	22

Table 3.1b, Boys: Percent in each stage of genital development on reaching PHV

3.1.2 Girls

3.1.2.1 Ages at Onset of Puberty, PHV and Menarche

The ages for pubertal onset, PHV and menarche are similar between our control group of girls and the UK references of Tanner. Menarcheal age from Tanner's Harpenden Growth study was acknowledged (Marshall and Tanner 1969) as being later than girls from more advantaged social circumstances who perhaps were more representative of the London population of the time. In fact Marshall and Tanner suggested an adjustment of 0.3 year to make their ages more representative. Age for menarche in the control cohort falls midway between the two quoted Tanner ages.

	Controls	Tanner	
B2	11.19 (1.19)	11.15 (1.10)	
PHV age	12.35 (0.83)	12.14 (0.88)	
Menarche	13.30 (0.99)	13.47 (1.02)	Harpenden
		13.00 (1.00)	So of England

Table 3.1c, Girls: Ages at Onset of Puberty, PHV and Menarche, mean (sd)

3.1.2.2 Tempo of Growth

Observations in Table 3.1d on the girls in the two cohorts in terms of pubertal development on achieving menarche and PHV show that there is close agreement in the distribution of breast stages at the time of occurrence of peak height velocity between the control cohort and the reference group.

Breast stage		1	2	3	4	5
PHV	Controls	3	28	56	13	0
	Tanner	0	26	51	23	0
Menarche	Controls	0	4	39	40	17
	Tanner	0	1	26	62	11

Table 3.1d, Girls: Percent in each stage of breast development on reaching PHV and menarche

The distribution for breast stage attainment at menarche, however, varies between the two; it is spread more evenly between stages 3 and 4 in the control cohort compared to the predominance in stage 4 for the reference group (similar to the differences in the distribution of the boys of genital stages at time of PHV).

*3.2 Control cohort, T1D cohort and the Tanner Reference**

This section is a comparison of the control and T1D cohorts and the Tanner references have been left in for interest not analysis.

3.2.1 Introduction

In the Chard study of the control children, there were initially 54 boys and 71 girls enrolled. The duration of follow-up, median (range) in both boys and girls is 6.4 (0.5-6.4) y. PHV was ascertained in 24 boys and 39 girls and reliable menarcheal ages are available for 47 girls. Data on final height was not examined since the children all moved school between 16 and 17 years of age. In the longitudinal study of 52 children with T1D (27 boys, 25 girls), the duration of follow-up, median (range), in girls is 5.9 (1-8.4) y and in boys 6.3 (2.1-8.9) y.

Peak height velocity (PHV) was established in 46 children (24 boys, 22 girls) and 35 (17 boys, 18 girls) were followed to final height (growth velocity 1 cm/yr or less). During the course of the study 22 girls achieved menarche.

3.2.2 Boys, Controls vs T1D

3.2.2.1 Ages at Onset of Puberty and PHV

(See Appendix for comment on ascertaining the age of pubertal onset).

There is a significant difference ($p<0.0005$) between the ages of pubertal onset between the control boys and those with T1D with the T1D boys being a year delayed (Table 3.2a). There is no statistical difference in the age at PHV between the 2 groups.

	Controls	T1D	Tanner
G2	11.28 (0.78)	12.32 (0.94)*	11.64 (1.07)
PHV age	13.82 (0.97)	14.20 (1.26)	14.06 (0.92)

Table 3.2a, Boys: Ages at Onset of Puberty and PHV, Controls vs T1D
Data are means (sd)

3.2.2.2 Tempo of Growth: Genital stage at the time of PHV

The distribution of genital stages at the time of PHV in the control boys is spread fairly evenly among stages 3, 4 and 5 whereas more than three quarters of the T1D boys are in stage 4 at this time (Table 3.2b).

Genitalia stage		1	2	3	4	5
PHV	**Controls**	0	4	25	33	38
	T1D	0	0	17	79	4
	Tanner	0	0	2	76	22

Table 3.2b, Boys: Percent in each genital stage at time of PHV

Looking at the actual numbers in each stage, comparing early (combining stages 2 and 3) and mid-late puberty (stages 4 and 5) and performing a chi square test: $\chi^2 = 4.46$ with 1 degree of freedom, $p \leq 0.05$. Control boys are in earlier puberty at PHV more often than boys with T1D (Table 3.2c).

Genitalia stage	Stage 2 and 3	Stage 4 and 5	Totals
Controls	12	12	24
T1D	5	19	24
Totals	17	31	48

Table 3.2c, Boys: Numbers in Early and Mid-late puberty at time of PHV

3.2.2.3 Ages at Reaching Different Stages of Puberty

T1D boys in this study are significantly older ($p<0.0005$) starting puberty than contemporary controls as already seen in table 3.2a. They are then a similar age at entering stage 3 but are older (but not statistically significant) for stages 4 and 5 (Table 3.2d).

		T1D	Controls	P
Puberty Stage 2	mean	12.32	11.28	<0.0005
sem		0.21	0.12	
Puberty Stage 3	mean	13.24	13.10	0.65
sem		.21	.23	
Puberty Stage 4	mean	14.11	13.56	0.09
sem		0.24	0.19	
Puberty Stage 5	mean	15.11	14.55	0.10
sem		0.30	0.15	

Table 3.2d, Boys: Ages at first reaching each stage of puberty

3.2.2.4 Time Intervals during Puberty

First appearance of G2 to first appearance of G5

The control boys take longer to go from the first appearance of stage 2 to the first appearance of stage 5 than T1D boys, median (range): 3.1 (1.1 - 4.6) vs 2.9 (1.4 - 5.2) y respectively. Although seemingly a small difference, it was statistically significant (P = 0.04).

From first appearance of G2 to PHV

The control boys take longer to go from the first appearance of stage 2 to PHV than the T1D boys, median (range): 2.55 (0.83-4.22) vs 1.84 (0.76-4.33) y respectively, P = 0.02.

3.2.2.5 Peak Height Velocity (PHV), age at PHV (PHVage), and PHV-SDS

There is no difference between the T1D and control boys in the magnitude of PHV, in the age of its occurrence or in the PHV-SDS adjusted for the age of occurrence (Methods) as demonstrated in Table 3.2e.

	BOYS	N	Mean	sd	SEM	P
PHVage	T1D	24	14.2	1.26	0.26	0.18
	Controls	24	13.82	0.97	0.20	
PHV	T1D	24	9.63	1.43	0.29	0.24
	Controls	24	10.2	1.46	0.30	
PHV-SDS	T1D	24	0.25	0.98	0.20	0.50
	Controls	24	0.46	1.12	0.23	

Table 3.2e, Boys: T1D vs Controls, PHVage, PHV, PHV-SDS

3.2.3 Girls

3.2.3.1 Ages at Onset of Puberty and PHV: Girls, Controls vs T1D

The T1D girls have a slightly earlier pubertal onset and menarche than the control girls but there is no statistical difference in the mean ages for the onset of puberty ($p = 0.13$) or menarche. There is, however, a significant statistical difference ($p = 0.05$) between the 2 groups for the age of PHV occurrence, the T1D girls have an earlier PHV. See Table 3.2 f:

	Controls	T1D	Tanner
B2	11.37 (1.00)	11.05 (0.60)	11.15 (1.10)
PHV age	12.35 (0.83)	11.92 (0.69)*	12.14 (0.88)
Menarche	13.26 (1.02)	13.21 (0.65)	13.0 (1.00)[a]
			13.47 (1.02)[b]

Table 3.2f, Girls: Ages at Onset of Puberty, PHV and Menarche, Controls vs T1D. Means (sd) (a=Southern England, b=Harpenden; as in section 3.1)

3.2.3.2 Tempo of Growth: Breast stage at PHV and Menarche

PHV: Both control and T1D cohorts have more than 80% in breast stages 2 and 3 at the time of PHV. The T1D girls, however, have a higher percentage (50%) at stage 2. The Tanner distribution is similar to the controls.

Breast stage		1	2	3	4	5
PHV	Control	3	28	56	13	0
	T1D	0	50	36	14	0
	Tanner	0	26	51	23	0

Table 3.2g, Girls: Percent in each breast stage at time of PHV

Using the absolute numbers and combining stages 2 and 3 (early puberty), a chi square analysis, χ^2 = 1.32 with 2 degrees of freedom, showed no significant difference between the controls and T1D girls in the stages of puberty at PHV (Table 3.2h).

Breast stage	Stage 1	Stage 2&3	Stage 4	Totals
Controls	1	33	5	39
T1D	2	17	3	22
Totals	3	50	8	61

Table 3.2h, Girls: Numbers in breast stages at time of PHV

Menarche: More than ¾'s of the girls in both the T1D and control cohorts (79% for controls and 86% for T1D) are in either breast stages 3 and 4 for menarcheal age; however, the T1D cohort is shifted to the right with 68% in stage 4 compared to 40% in the controls. The Tanner references are similar to the T1D cohort (See table 3.2i).

Breast stage		1	2	3	4	5
Menarche	Controls	0	4	39	40	17
	T1D	0	0	18	68	14
	Tanner	0	1	26	62	11

Table 3.2i, Girls: Percent in each breast stage at time of Menarche

A chi square analysis between the two cohorts using the numbers in breast stages 2/3 compared to 4/5 at the time of menarche: χ^2 = 3.07, 1 degree of freedom, showed no significant difference between the two groups in the stage of puberty at the time of menarche (Table 3.2j).

Breast stage	Stage 2/3	Stage 4/5	Totals
Controls	21	26	47
T1D	5	17	22
Totals	26	43	69

Table 3.2j, Girls: Numbers in breast stages at the time of Menarche

3.2.3.3 Ages at Reaching Different Stages of Puberty

The T1D girls enter puberty at a slightly younger age than the controls and reach stage 3 at a very similar age and then are slightly younger at stage 4 but none of these are statistically significant. It is not until stage 5 where there is a significant difference (p=0.001) in the age of attainment with the T1D girls being a year later.

		T1D	Controls	P
Puberty Stage 2	**mean**	11.05	11.37	0.32
sem		0.15	0.15	
Puberty Stage 3	**mean**	11.99	12.00	0.97
sem		0.20	0.17	
Puberty Stage 4	**mean**	12.75	13.06	0.39
sem		0.19	0.18	
Puberty Stage 5	**mean**	14.71	13.66	0.001
sem		0.28	0.16	

Table 3.2k, Girls: Ages at first reaching each stage of puberty

3.2.3.4 Time Intervals

First appearance of B2 to first appearance of B5

The girls with T1D take significantly longer (P<0.0005) to go from the first appearance of breast stage 2 to the first appearance of breast stage 5 than control girls; median (range): 4.1 (1.8 - 5.9) vs 2.5 (1.5 - 4.9) y respectively.

B2 to Menarche

Girls with T1D take longer than control girls to go from the first appearance of breast stage 2 to menarche, median (range): 2.2 (1.2 - 3.4) vs 1.8 (0.3 - 4.3) y but this did not reach statistical significance (P=0.15).

Menarche to B5

Girls with T1D take significantly ($p<0.0005$) longer to go from menarche to the first appearance of breast stage 5 than control girls; median (range): 1.1 (-.3-3.2) vs 0.7 (-0.8-1.3) y respectively.

B2 to PHV

Interestingly there is absolutely no difference in the median duration B2-PHV between the two cohorts (T1D vs controls respectively): 0.95 (-0.3 – 2.7) y vs 0.95 (-0.7 – 3.1) y. Note the negative values in the range since 2 girls in each cohort appeared to have a PHV before B2 was documented.

PHV to Menarche

The girls with T1D take longer to go from PHV to menarche, median (range) 1.3 (0.5-2.3) y vs 0.9 (-1.0 – 2.0) y than the control girls. This difference did not reach statistical significance (P=.09).

3.2.3.5 Peak Height Velocity (PHV), Age at PHV (PHVage) and PHV-SDS

There is no difference between T1D girls and controls in the magnitude of PHV or in the PHV-SDS adjusted for age of occurrence. T1D girls, however, have their PHV at an earlier age ($p=0.05$) see Table 3.2l.

	GIRLS	N	Mean	sd	SEM	P
PHVage	T1D	22	11.92	0.69	0.15	0.05
	Controls	39	12.35	0.83	0.13	
PHV	T1D	22	7.67	1.11	0.24	0.87
	Controls	39	7.71	0.96	0.15	
PHV-SDS	T1D	22	-0.62	0.93	0.20	0.36
	Controls	39	-0.41	0.77	0.12	

Table 3.2l, Girls: T1D vs Controls, PHVage, PHV and PHV-SDS

3.3 Sex Differences

3.3.1 PHV Magnitude (cm/yr): T1D vs Controls (Tanner reference)

In both sexes, as previously seen, there was no significant difference between the two groups in the magnitude of PHV (Tables 3.2 e and l). There was, however, a distinct sex difference between subjects with T1D and controls when compared with Tanner references for velocity. The age adjusted SDS for PHV was significantly reduced in the T1D girls (-0.62 ± 0.93, p = 0.001) but not in the boys (0.25 ± 0.98, p = 0.11). It is interesting to note that both the T1D and control girls had blunted spurts (7.67 ± 1.11cm/yr and 7.71 ± 0.96 cm/yr respectively) that were significantly lower than Tanner (8.4 ± 0.9 cm/yr, p < 0.05). These data are represented in Figure 3.3a, which also demonstrates the variation in the age at which PHV was attained.

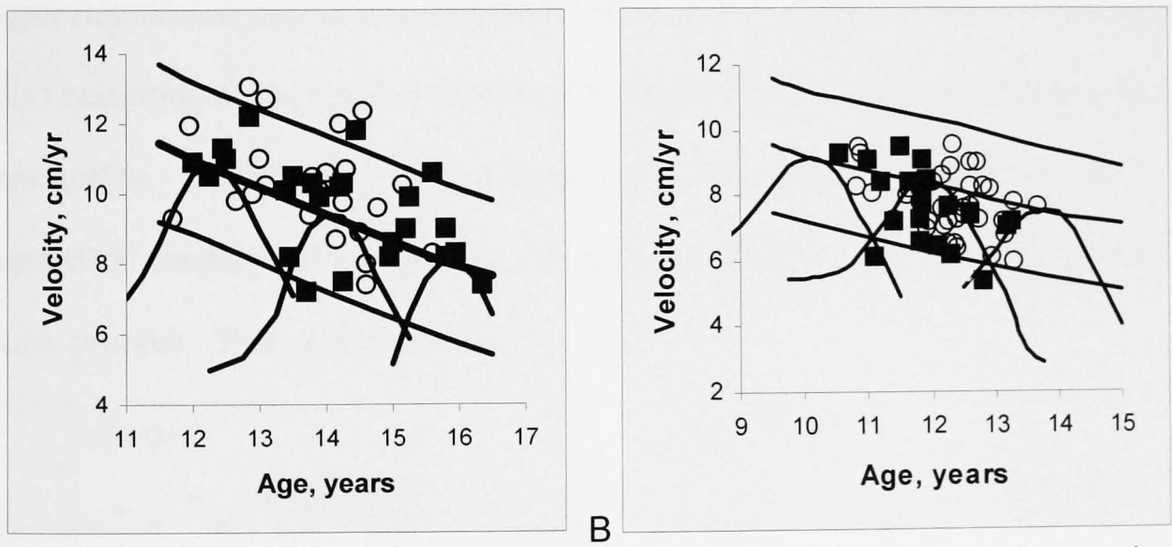


Figure 3.3a, Boys(A) and Girls(B): T1D (■) and Control (o), PHV plotted on the UK velocity references showing the 50th centile curves for early, average and late age at PHV. The straight lines are the 3rd, 50th and 97th centiles for the magnitude

3.3.1 Regression of PHV (cm/yr) on Age of PHV

Girls	N	B	SEM
T1D	22	-0.81	0.31
Controls	39	-0.58	0.16
Tanner		-0.47	0.17
Boys			
T1D	24	-0.63	0.20
Controls	24	-0.58	0.29
Tanner		-0.77	0.21

Table 3.3a: Regression of PHV, cm/yr against PHV age

The details of the regression of PHV (cm/yr) against the age of its occurrence (Table 3.3a) are negative as expected. Thus the earlier spurting child has a more vigorous peak height velocity. It is interesting to note that the control girls and boys of this study have an identical regression coefficient (B= - 0.58) whereas Tanner's Harpenden data showed the boys to have a greater regression than the girls (- 0.77 vs - 0.47 respectively). The T1D girls had a larger regression coefficient than the T1D boys (- 0.81 vs - 0.63) and therefore a spurt occurring a year later in these girls would be 0.81 cm/yr less than one a year earlier. In fact the control children (both sexes) and T1D boys all had regression coefficients of a similar magnitude whereas that of the T1D girls was much greater. This is illustrated in Fig 3.3b (1-4).

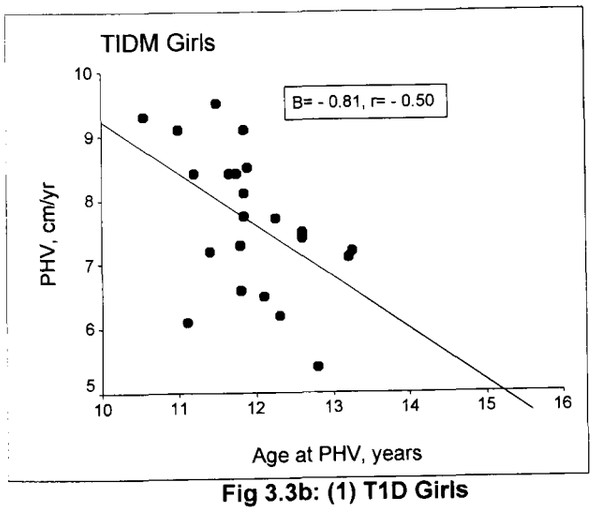
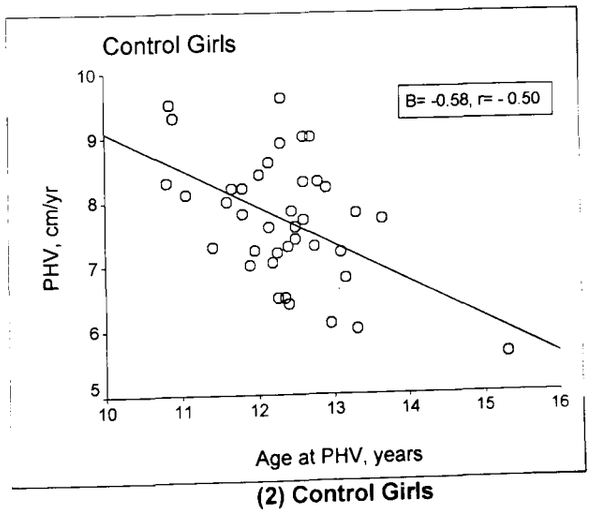
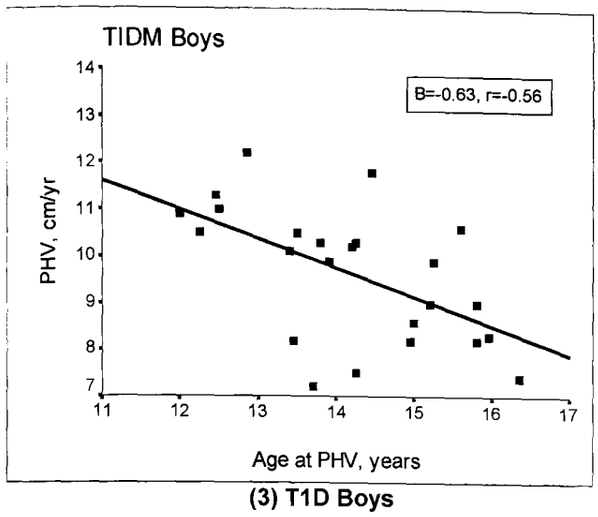


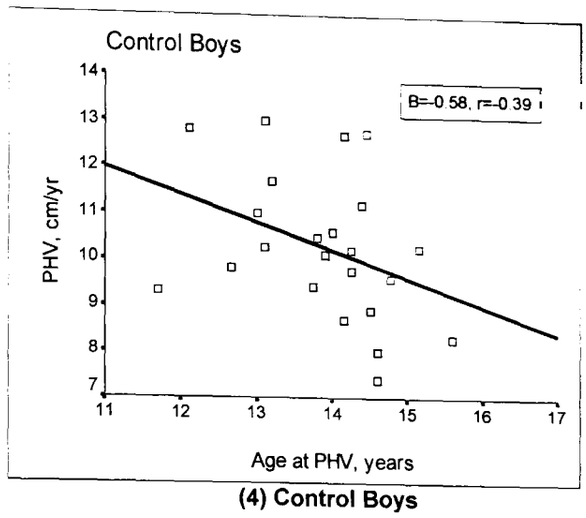
Fig 3.3b: (1) T1D Girls



(2) Control Girls



(3) T1D Boys



(4) Control Boys

3.3.3 Onset of Puberty, T1D: Bone Age vs Chronological Age

A subset of T1D children had bone ages (TW2RUS) available at the onset of puberty. The bone ages of the boys were slightly younger compared to their chronological ages, however, this did not reach statistical significance, mean (sd); 11.75 (1.07) y compared to 12.10 (0.71) y, p = 0.20. In the girls there was an advanced bone age, which was statistically significant, of 11.48 (1.01) y relative to the chronological age of 10.93 (0.86) y, p=0.04.

	Age	T1D	P
Boys	CA	12.10 ± 0.71	
n = 18	BA	11.75 ± 1.07	NS
Girls	CA	10.93 ± 0.86	
n = 19	BA	11.48 ± 1.01	0.04

Table 3.3b: Chronological and Bone Ages at Puberty Onset, Means,sd.

3.3.4 PHV and Menarche, T1D: Bone Age vs Chronological Age

PHV:

There was no difference between the bone age and chronological age of the T1D boys at age of PHV whereas the girls with T1D had an advanced bone age compared to their chronological age at PHV although this did not reach statistical significance (Table 3.3c).

	Age	T1D	P
Boys	CA	14.00 ± 1.29	
n = 19	BA	13.98 ± 0.70	NS
Girls	CA	11.96 ± 0.71	
n = 19	BA	12.28 ± 0.85	NS

Table 3.3c, Ages on reaching PHV: CA=chronological age, BA=TW2RUS bone age. $\bar{x}$ (sd)

Menarche:

The chronological age at menarche (N=19) was 13.23 ± 0.69y compared to a bone age of 13.42 ± 0.60 y and this difference was not statistically significant.

3.3.5 Height SDS changes

Height SD scores were calculated for boys and girls in both cohorts at the time of first appearance of puberty stage 2 and stage 5 and at the time of PHV. The results are in Table 3.3d:

BOYS	T1D	Controls
Stage 2	-0.28 (1.10)	0.06 (1.02)
PHV	-0.15 (1.15)	0.43 (0.94)
Stage 5	0.18 (1.00)	0.41 (0.93)
GIRLS		
Stage 2	0.10 (0.93)	-0.14 (0.89)
PHV	0.20 (0.98)	0.10 (0.81)
Stage 5	0.30 (1.05)	0.58 (0.68)

Table 3.3d, Boys and Girls: T1D and Controls, Ht SDS, $\bar{x}$ (sd)

There is no statistically significant difference between controls and T1D children in any of these Ht SD scores at the three points observed.

3.3.6 T1D, Height SDS changes from Diagnosis to Final Height:

The pattern of change in height SDS in T1D boys and girls at various points from diagnosis to final height was different (Table 3.3e).

At diagnosis the girls were tall relative to the UK references. However at final height they were marginally short (height SDS = -0.037 and the loss of height SDS from diagnosis to final height was statistically significant (p = 0.014). The boys were slightly taller than the reference at diagnosis (height SDS = 0.235) but by the onset of puberty their mean HtSDS of 0.06 (1.02) was similar to their final height SDS, -0.04 (0.78). Correcting the subjects' final height SDS to allow for the midparental height SDS, the girls were significantly short (p = 0.005) whereas the boys were not.

T1D	Diagnosis	PHV	Final Ht	Midpt HtSDS	Final HtSDS corrected for midpt ht
Boys	0.24	-0.15	-0.04	0.16	-0.17
Girls	0.81	0.20	-0.04*	0.40	-0.36 ϒ

Table 3.3e: Mean Height SDS Changes from Diagnosis to Final Height

*** P=0.014 From diagnosis to final height**

ϒ P=0.005 For final height corrected for parents' heights

3.3.7 T1D, Insulin dose and HbA1c before and after PHV:

Changes in insulin dose and HbA1c in the years before and after PHV are summarised in Table 3.3f. Mean HbA1c levels were lower in the girls during the years leading up to PHV but increased in both sexes during pubertal growth. There was no significant difference in HbA1c at PHV in boys, 9.72 (1.96)% vs. 8.86 (1.47)%, in girls even though there were differences in the PHV-SDS between the sexes. The maximum insulin dose during the growth spurt was similar in both sexes (1.14 u/kg/day in boys and 1.15 u/kg/day in girls) although there were differences in the time at which this dose was achieved (at PHV in boys and 2yrs after PHV in girls). Insulin dose at PHV was not significantly

different between the sexes: 1.14 (0.17) u/kg/day in boys and 1.10 (0.26) u/kg/day in girls.

Years from PHV		-3	-2	-1	0	1	2	3
Insulin dose	Boys	0.92 (0.20)	1.01 (0.18)	1.04 (0.16)	1.14 (0.17)	1.13 (0.15)	1.08 (0.14)	1.04 (0.14)
	Girls	0.78 (0.19)	0.87 (0.29)	0.95 (0.24)	1.10 (0.26)	1.13 (0.19)	1.15 (0.21)	1.03 (0.19)
HbA1c	Boys	9.07 (1.82)	9.73 (1.55)	9.73 (1.67)	9.72 (1.96)	9.64 (1.55)	9.89 (2.20)	9.88 (2.36)
	Girls	8.42 (1.31)	8.00* (1.93)	9.11 (1.01)	8.86 (1.47)	9.73 (1.69)	9.73 (1.66)	10.15 (1.88)

Table 3.3f: Insulin dose (u/kg/day) and HbA1c (%) in the Years Before and After PHV. Data are mean (sd). * p<0.027 between boys and girls for HbA1c at PHV-2 years.

3.3.8 T1D, Insulin dose and HbA1c at PHV

HbA1c correlated negatively with PHV-SDS (r = -0.25, Fig 3.3c) in both sexes. This was, however, only significant in the boys; r = - 0.41, p = 0.05 (the girls: r = - 0.24, p = 0.3. There was no relationship between insulin dose and PHV-SDS.

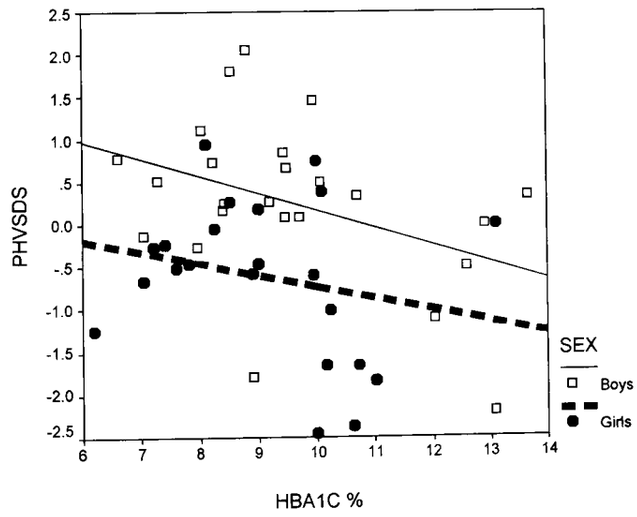


Figure 3.3c, T1D Girls and Boys: PHVSDS and HbA1c

3.4 Summary

The normal cohort that has been used as a control group is contemporaneous in time with the T1D cohort and from a similar social environment. These control subjects of both sexes were found to have similar ages to the Marshall and Tanner data for the onset of puberty, timing of their PHV and age of menarche.

Although the majority of boys in both cohorts experienced PHV in late puberty, 29% of the control boys were in early to mid puberty compared to Marshall and Tanner's predominantly at stage 4. The tempo of growth in the girls was similar at PHV but differed with respect to menarche in that an equal percent were in stages 3 and 4 as opposed to Marshall and Tanner's data predominantly at stage 4.

Puberty staging is subjective and it must be remembered that the Tanner stages were determined on photographs whereas the ratings on the control subjects for this study were done in person. Both genital and breast stage 4, however, have very definite criteria so this may reflect a real difference between these 2 groups. It is difficult to categorically state whether this reflects a difference in the tempo of pubertal maturation between the two or highlights the problems of different techniques and different observers. However, as previously stated, the mean age of PHV in both sexes and menarcheal age is similar between the two groups. This investigator has therefore concluded that the Chard sample is reasonably well validated by the Tanner references and the differences are primarily related to the possible earlier tempo of growth with the control boys having an earlier pubertal onset with PHV occurring at stages 3, 4 and 5 not primarily in stage 4 and in the control girls, menarche appears equally in stages 3 and 4 not predominantly in stage 4.

The onset of puberty was a year later in T1D boys compared to controls. Control boys were found to be in an earlier stage of puberty at PHV and they take longer to go through puberty. There was no difference for PHV, PHV-SDS or PHVage.

The onset of puberty and menarcheal age was slightly earlier in T1D girls (not statistically significant). At the onset of puberty, some of the T1D girls had an

advanced bone age relative to their chronological age. By the attainment of stage 5 however, they were significantly older than controls having taken longer to go through puberty. There was no significant difference in the tempo of growth relating to breast stages at the time of PHV or menarche between the 2 groups.

Although there was a difference in the age at PHV with T1D girls being 0.4 year younger, there was no difference in PHV or PHV-SDS. It is interesting to note that both study cohorts of girls have low PHV-SDS's (T1D: - 0.62, controls: - 0.41). We could speculate if this sample of girls were unusual or was it too small a sample or perhaps the 6 monthly visits did not allow as accurate an assessment as 3 monthly would have allowed.

The regression of PHV on age at PHV was negative as expected; it was interesting to see that the T1D girls had the steepest slope indicating greater loss of PHV magnitude with each year later than either the control girls or either cohort of boys.

T1D girls have a statistically significant advanced bone age at the onset of puberty but not at PHV or menarche.

In the T1D cohort, height SDS changes through puberty demonstrated that the girls were tall at the time of diagnosis but ended up relatively short even when their parents' heights were taken into account. This was not so for the boys.

At the time of PHV, both T1D boys and girls had similar doses of insulin (u/kg/d) and their maximum dose during the growth spurt was the same although the timing was different, the boys had their maximum dose at PHV and the girls at 2 years after PHV. PHV-SDS was negatively related to HbA1c% although this was only significant in the boys. There was no relationship between insulin dose and PHV-SDS.

Chapter 4. Body Composition in T1D and Control Cohort

4.1 Introduction

As children grow and enter puberty, characteristic sex specific gains in fat mass and fat free mass become more evident. Although both sexes put on weight as they grow, there is a difference in the partitioning (Wells 2007). Girls tend to gain far more fat mass relative to fat free mass and continue this increase into late puberty. Boys have a dramatic increase in fat free mass and a decrease in percent body fat as they go through puberty. Adolescents of both sexes with T1D have been seen to have an increase in weight (or BMI) far more than their age related peers (see chapter 1). This excessive weight gain is often more evident in girls.

To compare the body composition changes by age in the two cohorts here, an analysis of covariance was used to look at the data longitudinally by entering the child's identifier as a fixed factor. This examines the association between variables within subjects by fitting parallel lines with a common slope for all subjects. Inter-subject variation is shown by differences in the constants and the regression coefficient describes the associations between variables (Bland and Altman 1995).

When comparing the cohorts by puberty stage, the mean of the variable in question was calculated for each subject in each stage (since children were often seen more than once in a puberty stage), so that an individual appears only once in any puberty stage. Differences were examined by Mann Whitney *U* non-parametric test.

MLwiN statistics were provided by Dr Ong for the overall BMI and percent body fat comparisons.

4.2 Boys

4.2.1 BMI By Age

BMI increased with age in both groups of boys: T1D, B=0.773 ± 0.024, p<0.0005; controls, B=0.681 ± 0.026, p<0.0005. Boys with T1D had a greater BMI than controls throughout the age range studied; overall they had 1.46 ± 0.55 kg/m^2 higher BMI than the control boys (MLwiN).

4.2.2 BMI By Puberty Stage

Boys with T1D had higher BMI's at all puberty stages compared to control boys and at each stage this was significant (Fig 4.2a).

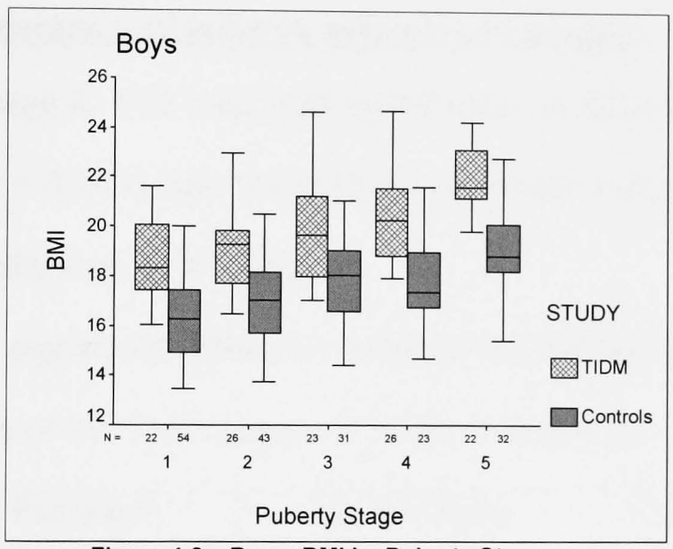


Figure 4.2a, Boys: BMI by Puberty Stage

	1	2	3	4	5
P	<0.0005	<0.0005	0.007	<0.0005	<0.0005

4.2.3 Body Composition by Age

Percent body fat increased with age in the control boys (B = 0.019 ± 0.008, p = 0.03) but decreased in the boys with T1D (B = - 0.047 ± 0.008, p<0.0005). Overall there was no significant difference between the T1D and control boys, 0.68 ± 1.27%, p = 0.6 (MLwiN).

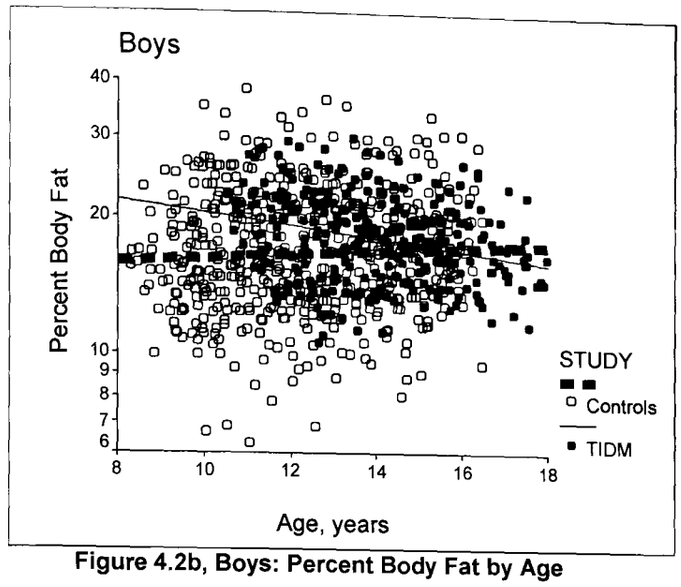


Figure 4.2b, Boys: Percent Body Fat by Age

4.2.4 Body Composition during Puberty

4.2.4.1 At first appearance of puberty stage 2 and stage 5

At the first sign of stage 2, T1D boys had significantly more fat mass (p = 0.006) and fat free mass (p = 0.001) than control boys and more percent body fat which almost reached significance (p = 0.055).

By stage 5, the only significant difference between the two groups was in the greater fat free mass of the T1D boys (p<0.0005), Table 4.2a.

	Fat mass	Fat free mass	Percent BF
Stage 2			
Controls	5.25 (2.28-15.93)	29.54 (21.42-37.67)	15.58 (8.67-29.95)
T1D	7.63 (4.86-11.35)	32.81 (28.94-39.95)	18.98 (13.65-22.81)
p	0.006	0.001	0.055
Stage 5			
Controls	8.63 (4.53-20.56)	44.70 (37.32-50.22)	16.38 (9.48-30.69)
T1D	10.15 (6.69-16.08)	49.48 (42.46-58.30)	16.38 (12.40-24.14)
p	0.068	<0.0005	NS

Table 4.2a, Boys: Fat mass, fat free mass and percent body fat at stage 2 and stage 5 Median (range)

4.2.4.2 Changes During Puberty

The control boys had no significant change in percent body fat analysed by puberty stage although a slight loss was seen (Table 4.2b). This is in contrast to the analysis by age where an increase was observed. However, the decrease in percent body fat in boys with T1D was still evident by pubertal stage analysis and this decrement was significantly different to that of the control boys ($p = 0.008$).

	T1D	Controls	P
Fat Mass, kg	3.5 ± 0.53	4.7 ± 0.77	0.2
% Body Fat	-3.7 ± 0.78	-0.2 ± 0.96	0.008
Fat-Free Mass, kg	24.0 ± 1.1	21.9 ± 0.59	0.1

Table 4.2b, Boys: Change in fat mass, percent body fat and fat free mass during puberty. Means ± SEM

4.3 Girls

4.3.1 BMI by Age

BMI increased in girls with age: T1D, B=1.0005 ± 0.027, $p<0.0005$; controls, B=0.895 ± 0.031, $p<0.0005$ and was higher in the girls with T1D compared to the control girls throughout the age range studied. Overall, T1D girls had 1.45 ± 0.69 kg/m^2 higher BMI than control girls (MLwiN).

4.3.2 BMI by Puberty Stage

In the girls (Fig 4.3a), a significant difference in BMI's between subjects with T1D and controls was seen but was not as great as in the boys.

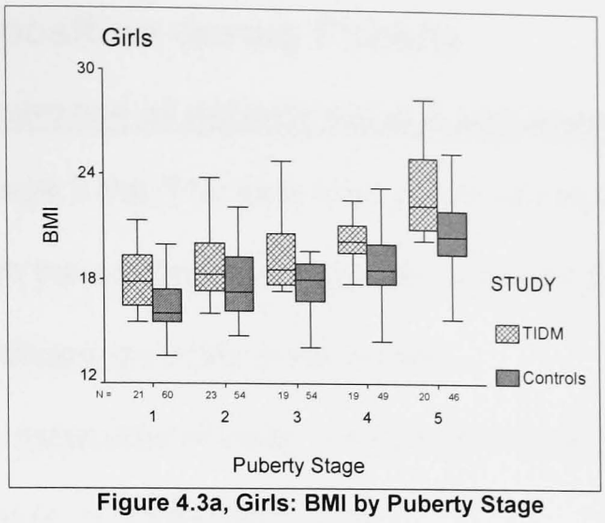


Figure 4.3a, Girls: BMI by Puberty Stage

Puberty stage	1	2	3	4	5
P	0.001	0.04	0.014	0.006	0.001

4.3.3 Body Composition by Age

In both girls with T1D and control girls, percent body fat increased with age: B = 0.127 ± 0.007, p<0.0005 and B = 0.180 ± 0.012, p<0.0005 respectively.

Overall, girls with T1D had a higher percent body fat than the control girls by 3.2 ± 1.0%, p = 0.002 (MLwiN).

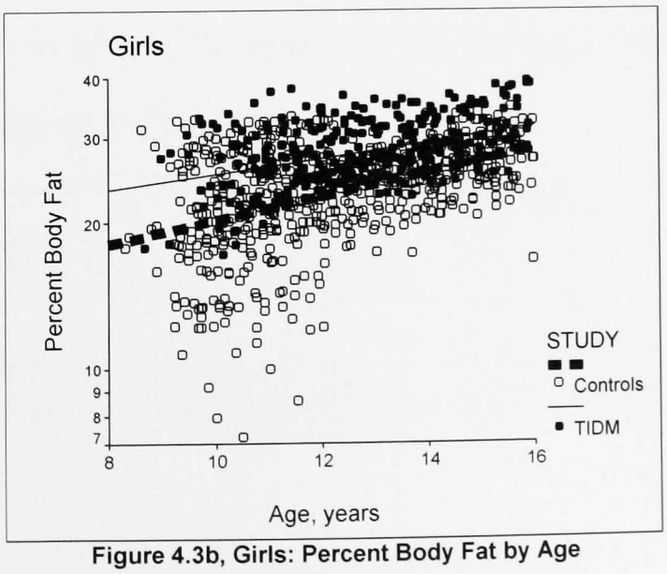


Figure 4.3b, Girls: Percent Body Fat by Age

4.3.4 Body Composition during Puberty

4.3.4.1 At first appearance of puberty stage 2 and stage 5

At the first sign of stage 2 the T1D girls have more fat mass, fat free mass and percent body fat than the control girls, however, it is only the differences in the fat mass that is significant (p=0.04) at this stage.

By stage 5, all three measures of body composition are greater in the T1D girls and highly significant (p<0.0005) Table 4.3a.

	Fat mass	Fat free mass	Percent bf
Stage 2			
Controls	8.56 (5.76-15.63)	26.17 (20.38-33.97)	24.81 (19.76-32.60)
T1D	9.83 (6.22-19.43)	28.94 (21.58-36.27)	26.13 (22.38-34.89)
p	0.04	0.087	0.087
Stage 5			
Controls	12.86 (8.60-22.42)	36.17 (30.89-42.28)	26.29 (20.32-34.66)
T1D	17.01 (13.95-26.85)	40.69 (34.37-47.55)	30.30 (26.34-38.58)
p	<0.0005	<0.0005	<0.0005

Table 4.3a, Girls: Fat mass, fat free mass and percent body fat at stage 2 and stage 5

4.3.4.2 Changes During Puberty

During puberty girls with T1D gained more body fat than control girls (p = 0.04), however, there was no difference between the 2 groups in the gains of fat free mass or percent body fat (Table 4.3b).

	T1D	Controls	P
Fat Mass, kg	10.7 ± 1.03	8.2 ± 0.41	0.04
% Body Fat	8.1 ± 1.19	7.6 ± 1.08	0.8
Fat-Free Mass, kg	13.7 ± 0.81	13.6 ± 0.67	0.9

Table 4.3b,Girls: Change in fat mass, percent body fat and fat free mass during puberty. Means ± SEM

4.4 Body Composition and Leptin during Puberty

4.4.1 Boys

In both T1D and control boys, leptin levels decreased with age: T1D, B = - 0.040 ± 0.015, p = 0.01; controls, B = - 0.063 ± 0.01, p<0.0005.

Fat free mass was negatively associated with leptin in both control, B = - 0.50 ± 0.08, p < 0.0005 and T1D boys, B = - 0.45 ± 0.14, p < 0.002 (Fig 4.4a).

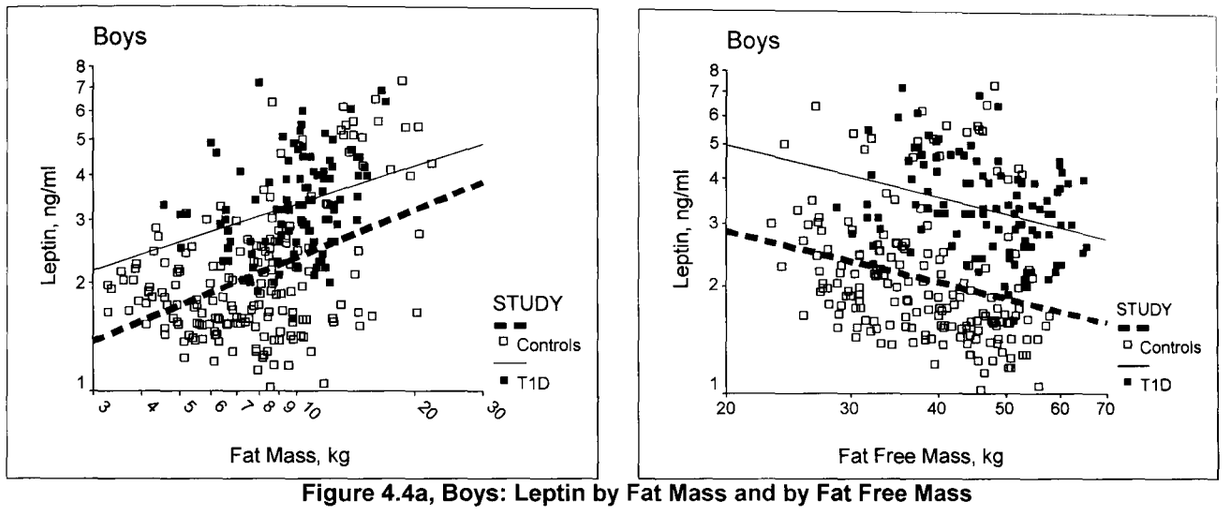


Figure 4.4a, Boys: Leptin by Fat Mass and by Fat Free Mass

Although not statistically significant, it can be seen that for any level of fat mass, leptin levels are again higher in the T1D boys than control boys.

When both fat mass and fat free mass were entered into the same model, fat free mass was negative allowing for levels of fat mass: B = - 0.89 ± 0.13, p < 0.0005 (controls) and B = - 0.98 ± 0.18, p < 0.0005 (T1D) while fat mass was positive adjusting for fat free mass in both controls and T1D boys respectively: B = 0.35 ± 0.09, p < 0.0005 and B = 0.71 ± 0.17, p < 0.0005.

4.4.2 Girls

Leptin levels increased with age in both girls with T1D and control girls: Type 1, B = 0.179 ± 0.02, p<0.0005; controls, B = 0.143 ± 0.012, p<0.0005.

Significant positive associations are seen with both fat mass and leptin and fat free mass with leptin in T1D and control girls, $p < 0.0005$:

Leptin and Fat Mass	B	se	p
Controls	0.59	0.06	<0.0005
T1D	1.26	.14	<0.0005
Leptin and FFM			
Controls	1.40	0.14	<0.0005
T1D	2.13	0.30	<0.0005

Table 4.4, Girls: Fat Mass and Fat Free Mass associations with Leptin

Considering leptin in relation to body fat and fat free mass, the graphs below clearly show that leptin levels are higher for all levels of both fat mass and fat free mass in the T1D girls relative to control girls.

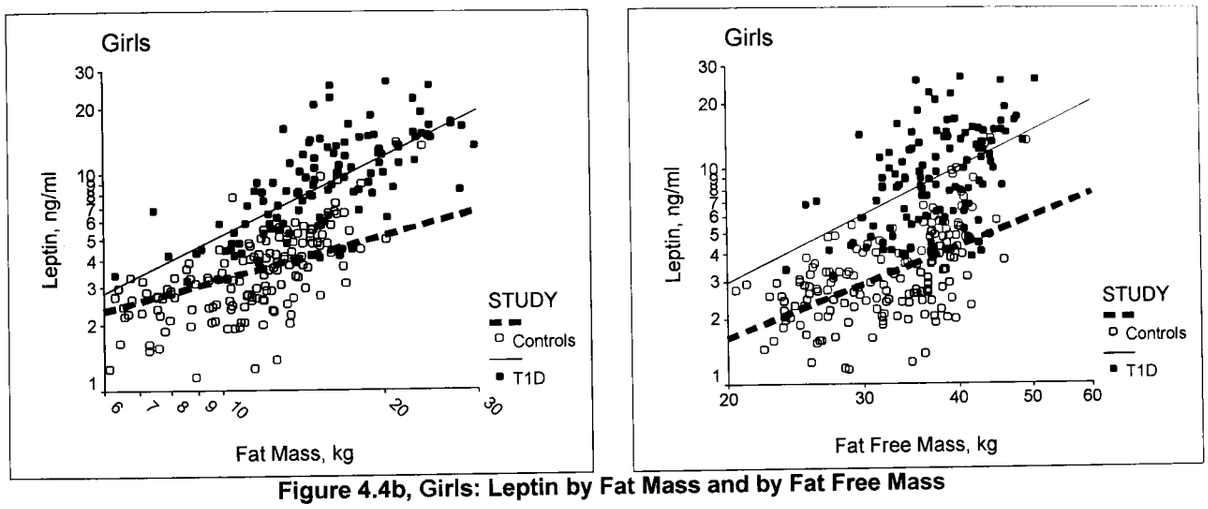


Figure 4.4b, Girls: Leptin by Fat Mass and by Fat Free Mass

Although previously fat mass and fat free mass had been entered into the same model (Ahmed et al. 1999; Ahmed et al. 2001), I have subsequently realised that they are highly correlated in both cohorts of girls, $r = 0.88$ and 0.81 (T1D and controls respectively) and since these close associations may make the model unstable I have omitted those results here.

In T1D girls, from puberty stage 1 to 4, total daily insulin dose adjusted for percent body fat was positively related to leptin levels, $B = 0.006 \pm 0.003$, $p = 0.05$. No such relationship was seen in the boys, $p = 0.1$.

4.5 Summary and Discussion

In all the subjects, BMI increases with both age or puberty stage and the T1D children, especially the boys, have a greater BMI than controls throughout the study period. This is in agreement with numerous previous reports (Gregory et al. 1992; Thon et al. 1992; Pitukcheewanont et al. 1995; Danne et al. 1997; Holl et al. 1998b; Domargard et al. 1999; Dabadghao et al. 2001; Holl et al. 2003; Ingberg et al. 2003; Codner et al. 2004; Luna et al. 2004). Percent body fat increased with age in control boys but decreased in the T1D boys however, overall there was no significant difference between the two. Analysis by puberty stage of percent body fat revealed no significant change in the control boys (although the decrease in the T1D boys was still evident). These different observations may relate to the different 'time-frame' of analyses by pubertal stage compared to chronological age since the pubertal change from the first stage 2 to the first stage 5 is shorter than the age time scale.

At the start of puberty, T1D boys had significantly more fat mass, fat free mass and percent body fat than control boys; however, by stage 5 the only significant difference was the greater fat free mass of the T1D boys. This is in contrast to the girls who at the start of puberty only had significant differences in fat mass (T1D girls greater) yet by stage 5 all 3 measures of body composition were significantly greater in the T1D girls. Thus the increases in BMI in girls was largely due to the recruitment of fat mass, whereas the higher BMI in boys was accompanied by a reduction in percent body fat and recruitment of fat free mass. These differences highlight important sexual dimorphism in both cohorts as well as the more pronounced effects in the diabetic subjects. It should perhaps be noted here that the data may have been better presented if the FM and FFM were normalised for height, ie adjusted for body size by dividing by ht^2

and presented as an index (kg/m^2) as recommended by Wells (Wells 2001). It may be something to consider for the future.

The mechanism of increased weight gain in T1D girls is not established although a relationship to insulin dose or frequency has been observed (Holl et al. 1994; Danne et al. 1997; Mortensen and Hougaard 1997). The conventional subcutaneous administration of insulin leads to peripheral hyperinsulinism which could lead to the increased adiposity (Dunger 1992).

The discovery of the hormone leptin (Zhang et al. 1994), which could regulate satiety, energy expenditure and weight gain has added another dimension to these relationships. Leptin is produced by adipocytes and feeds back through the hypothalamic receptors to regulate weight gain and energy expenditure (Rohner-Jeanrenaud et al. 1996). Leptin deficiency was originally thought to be a cause of obesity (Campfield et al. 1996), but this is unusual, paradoxically, obesity tends to be related to high leptin levels, suggestive of leptin resistance (Caro et al. 1996).

In general, reports on leptin levels in T1D children/adolescents have observed higher levels in T1D subjects compared to controls than would be expected for their degree of fat mass (Kamoda et al. 1998; Kiess et al. 1998; Luna et al. 1999; Bideci et al. 2002; Soliman et al. 2002; Morales et al. 2004) however Kirel et al reported lower levels in T1D children (Kirel et al. 2000) and three groups have found similar leptin levels between controls and T1D subjects (Verrotti et al. 1998; Myers et al. 2004; Karaguzel et al. 2006). Indeed in addition, Karaguzel et al found no difference in body composition measured by DXA between his T1D children and controls

Most attempts at intensification of insulin therapy have led to greater weight gains than that observed with more standard therapy (Anonymous 1988;

Reichard et al. 1991; Anonymous 1995; Danne et al. 1997; Mortensen and Hougaard 1997; Holl et al. 1998b). It is possible that the peripheral hyperinsulinaemia could lead to greater leptin secretion. Studies by Kolaczynski et al (Kolaczynski et al. 1996) showed that insulin did not regulate leptin acutely but that long term administration of insulin increased leptin levels both in vivo and in vitro and Tuominen (Tuominen et al. 1997) observed that chronically high levels of insulin in T1D young men led to higher leptin levels compared to controls but also suggested that unchanged leptin levels before and after a euglycaemic hyperinsulinaemic clamp meant that the T1D subjects were resistant to the acute insulin stimulus.

In spite of higher leptin levels at the start of puberty, girls with T1D gain more body fat during puberty compared to control girls. The term 'leptin resistance', however, needs to be used with caution as the role of leptin in the regulation of normal pubertal weight gain has yet to be confirmed (Ahmed et al. 1999) especially as the T1D boys also had elevated leptin levels and did not gain more fat mass but showed higher gains in fat free mass.

Chapter 5. Endocrine Changes during Puberty

5.1 Introduction

Numerous factors influence the pubertal growth and development of children including nutrition and body composition, psychological well-being, physical exercise, sleep, genetics and the hormonal environment. Growth hormone's action is hypothesized to take one of two routes: either to be mediated by IGF-I which then acts either as a classic endocrine hormone (the somatomedin hypothesis) or alternatively to act directly causing precursor cells to differentiate and produce IGF-I (the dual effector theory), this then signals the local generation of IGF-I that is responsible for the clonal expansion of the chondrocyte cell line (and thus growth). Insulin acts as an anabolic hormone increasing at puberty to compensate for the pubertal insulin resistance which then results in a decrease in IGFBP-1 and leads to an increase in the bioavailability of free IGF-I. As insulin increases, SHBG decreases which subsequently results in an increase in circulating sex hormones. GH pulse amplitude is increased by both testosterone and oestradiol (through aromatisation from testosterone) and it is thought that estrogens are more potent modulators of GH action than testosterone. Leptin has been shown to have a 'permissive' effect for the onset of puberty, levels of leptin increase with puberty in girls and decrease in boys. Although androgens are thought to influence the development of pubic and axillary hair, their role in the initiation of puberty remains contentious. Adequate levels of thyroid hormone are necessary for normal growth and pubertal development and circulating levels appear to decrease during early puberty.

All hormones considered here have been explored in relation to a developmental scale, i.e. years from peak height velocity and puberty stage rather than age. Age, however, was used in the initial data inspection and these age graphs are in Appendix 8.12.1. Ascertainment of age at PHV has been described in chapter 2 section 2.2.4.1 and for the plots by puberty stage; if a child was seen more than once in a puberty stage, the mean in each stage was calculated and each child appears only once in a stage.

Although 8 hormones have been measured in both cohorts, only those that appear to differ between the 2 groups are presented here. FT4 was originally considered (age graphs are in the Appendix), however, as can be seen in the table of geometric means by years from PHV (section 5.9), there is little difference between the two cohorts of either sex in levels of FT4 and so it was not considered further.

All statistical analyses have been done on logged hormone values and converted to geometric means in the tables for a more meaningful interpretation. Unlogged values are presented on the graphs of grouped data (either in years from PHV or puberty stages) as box plots with median and interquartile range for visual clarity and the hormone axes have been logged on scatter graphs, thereby keeping the real units, for ease of interpretation.

On the scatter graphs of maximal hormone levels in relation to PHV, the dotted lines on the y axis are to aid in the detection of the maximal value achieved and the dotted lines on the x axis to pinpoint the time of its occurrence.

5.2 DHEAS

5.2.1 Boys

5.2.1.1 DHEAS in relation to years from PHV

DHEAS levels were lower in T1D boys compared to controls and increased more slowly in relation to years before and after PHV. In a covariance analysis with log DHEAS as the dependent variable, years from PHV as a covariate and subject id as a fixed factor (to allow for the longitudinal nature of the data), B ± SEM: T1D boys, 0.04 ± 0.007, p <0.0005; and control boys, 0.14 ± 0.006, p<0.0005. Grouping the data in to whole year PHV groups (± 0.5 years), the two cohorts are compared in Figure 5.2a.

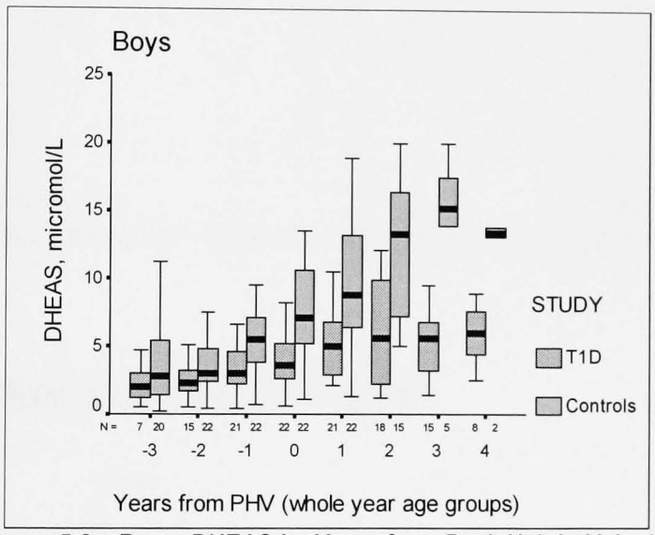


Figure 5.2a, Boys: DHEAS by Years from Peak Height Velocity

The data has been grouped in whole year PHV groups and is displayed as the median with the interquartile range (25^{th}-75^{th} centile) and the 'inner fence' as ± (1.5 (75^{thc}-25^{th}c)) from the edge of the boxes.

The geometric means of DHEAS by years from PHV and the significance between T1D and control boys are presented in the following table (Table 5.2a).

Boys	-2	-1	0	1	2	3
T1D	2.3	2.8	3.6	4.8	4.8	4.7
Controls	3.0	4.4	6.3	8.3	10.5	13.2
P	NS	0.04	0.009	0.006	0.003	0.004

Table 5.2a, Boys: DHEAS levels by Years from Peak Height Velocity (Geometric Mean)

These data show that from 1 year before PHV until at least 3 years after, T1D boys have significantly lower levels of DHEAS compared to control boys.

5.2.1.2 Maximal DHEAS levels in relation to PHV

The SPSS graphical method using Loess (Methods 2.2.4) allows a line of best fit through the individual data points. Figure 5.2b is a graph of the individual points plotted against years from PHV. The maximum value of 5.5micromol/L is achieved at 4.5 years after PHV in T1D boys and the curve plateaus at this value whereas in the control boys a value of 17 micromol/L is achieved at this time, but the data are too scanty to determine if that is the maximum.

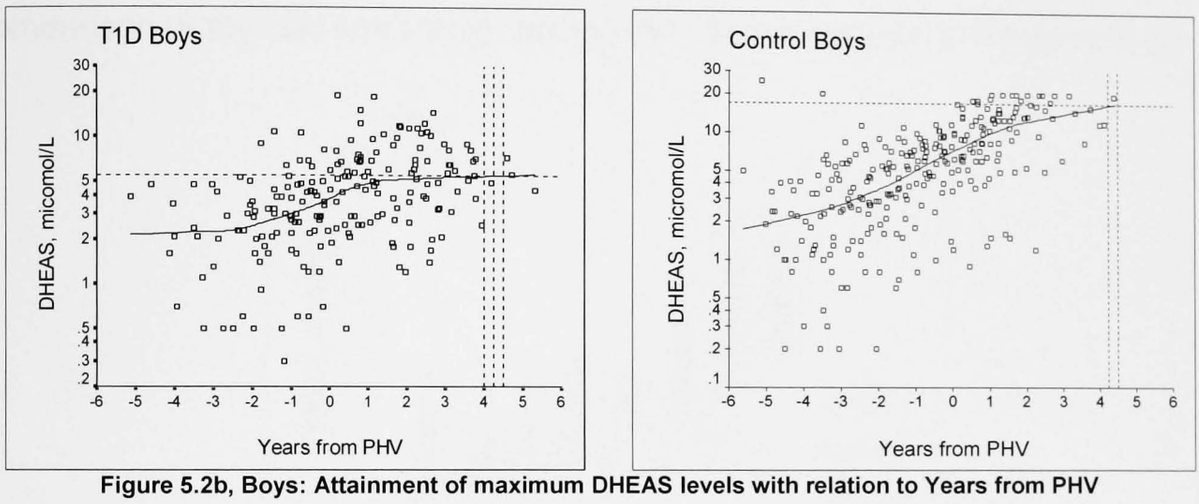


Figure 5.2b, Boys: Attainment of maximum DHEAS levels with relation to Years from PHV

5.2.1.3 DHEAS in relation to Puberty Stages

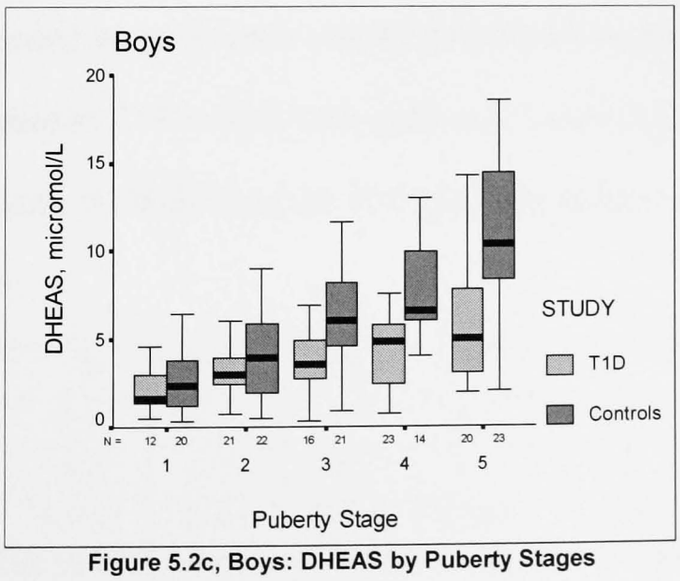


Figure 5.2c, Boys: DHEAS by Puberty Stages

Grouping the data by puberty stages, DHEAS levels were lower in T1D boys compared to controls at all puberty stages and were statistically significantly different at stage 3 ($p = 0.02$), stage 4 ($p = 0.003$) and stage 5 ($p<0.0005$).

5.2.2 Girls

5.2.2.1 DHEAS in relation to years from PHV

DHEAS levels were lower in T1D girls compared to controls and increased more slowly in relation to years before and after PHV. In a covariance analysis with log DHEAS as the dependent variable, years from PHV as a covariate and id as a fixed factor (to allow for the longitudinal nature of the data) B ± SEM: T1D girls, 0.03 ± 0.009, p = 0.004; and control girls, 0.10 ± 0.005, p<0.0005. A comparison of the two cohorts by whole year PHV groupings is in Figure 5.2d.

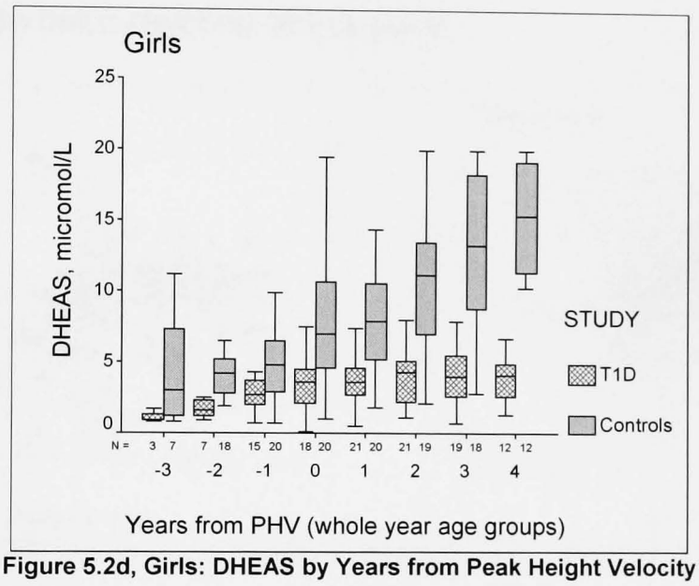


Figure 5.2d, Girls: DHEAS by Years from Peak Height Velocity

The data are presented as geometric means of DHEAS by years from PHV and the significance between T1D and control girls is in Table 5.2b. Statistically significant lower levels of DHEAS in the T1D girls are observed throughout the time period studied.

Girls	-2	-1	0	1	2	3
T1D	1.7	2.6	2.6	3.1	3.6	3.3
Controls	4.2	4.5	6.2	7.1	8.9	11.5
P	0.003	0.04	0.005	0.001	<0.0005	<0.0005

Table 5.2b, Girls: DHEAS levels by Years from Peak Height Velocity (Geometric Mean)

5.2.2.2 Maximal DHEAS levels in relation to PHV

The curve for DHEAS in relation to PHV was again explored using the Loess graphical method in SPSS.

The maximum value of DHEAS (Figure 5.2e) achieved by T1D girls is lower than in the control girls (4.0 vs 16.25 micromol/L) and occurs much earlier in relation to PHV (0.75 years after PHV) compared to 5.25 years after PHV in control girls. In the latter, the DHEAS may continue to increase; it is difficult to say if a plateau has been reached at this point.

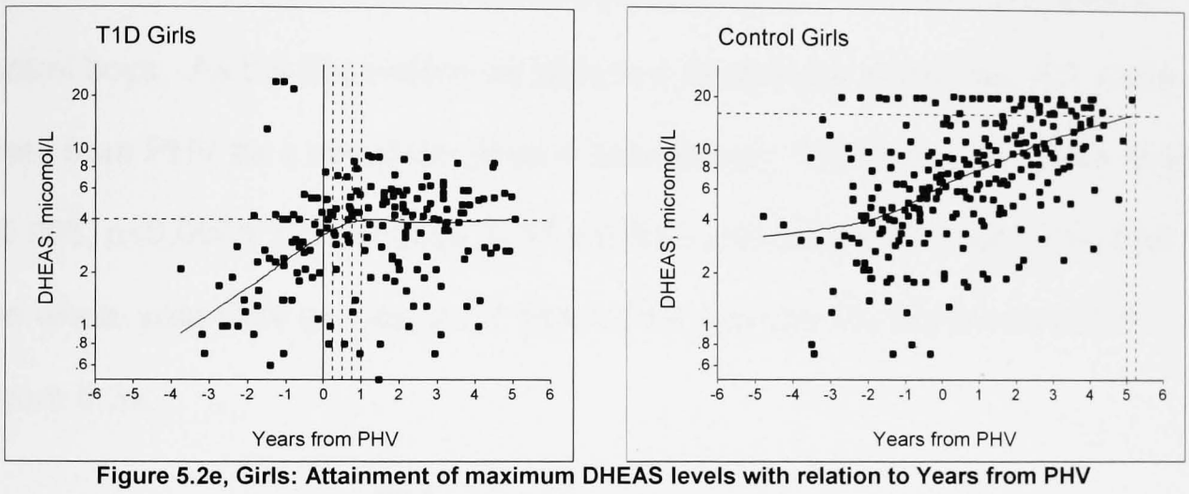


Figure 5.2e, Girls: Attainment of maximum DHEAS levels with relation to Years from PHV

5.2.2.3 DHEAS in relation to Puberty Stages

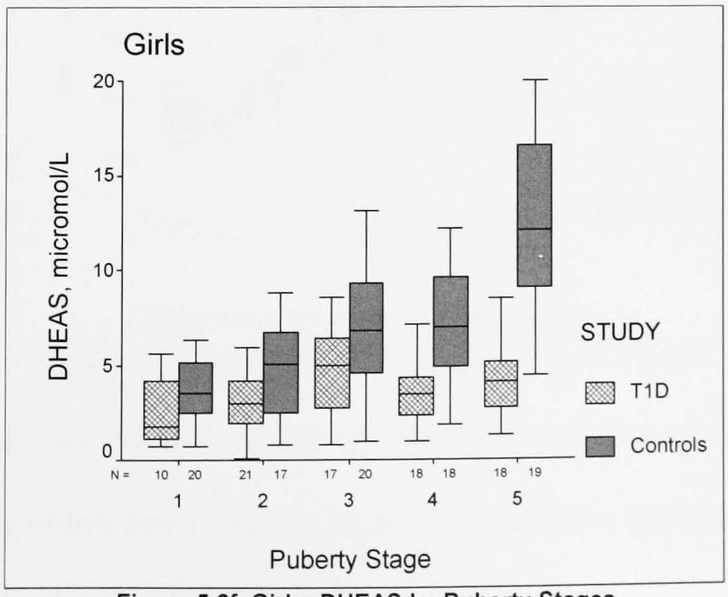


Figure 5.2f, Girls: DHEAS by Puberty Stages

Similarly to the boys, DHEAS levels were lower in T1D girls compared to controls at all puberty stages and this difference became statistically significant with the onset of puberty: stage 2, p=0.05; although stage 3 did not reach statistical significance (p=0.08); however, both stage 4 and stage 5 did, p=0.001 and p<0.0005 respectively.

5.3 IGF-I

5.3.1 Boys

5.3.1.1 IGF-I in relation to years from PHV

IGF-I levels were lower and increased more slowly in T1D boys compared to control boys. As the dependent variable in a covariance model, log IGF-I with years from PHV as a covariate (id as a fixed factor): T1D boys (B ± SEM), 0.04 ± 0.006, p<0.0005; control boys, 0.07 ± 0.003, p<0.0005). Grouping the data into whole year PHV groups (± 0.5 years); the two cohorts are compared in Figure 5.3a.

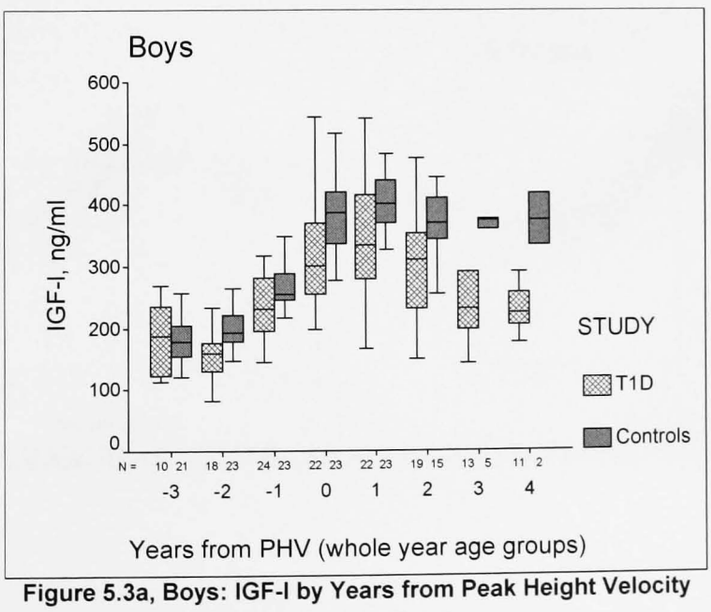


Figure 5.3a, Boys: IGF-I by Years from Peak Height Velocity

To statistically quantify the difference, the data are presented as geometric means of IGF-I by years from PHV in table 5.3a and the significance of the difference between the two cohorts is given.

Boys	-3	-2	-1	0	1	2	3
T1D	174.34	149.68	225.05	306.42	315.62	267.20	241.68
Controls	180.60	197.92	268.08	376.96	401.83	372.24	373.08
P	NS	<0.0005	.03	.002	.002	.02	.004

Table 5.3a: Boys: IGF-I levels (ng/ml) by Years from Peak Height Velocity (Geometric mean)

The levels of IGF-I were lower in T1D boys compared to controls from 3 years before to 3 years after PHV. This difference became significant 2 years before PHV and then remained so throughout the period studied.

5.3.1.2 Maximal Levels of IGF-I by Years from PHV

The Loess graphical method (SPSS) was used to explore if and at what time the curve of IGF-I reached a maximum/plateau in relation to PHV (Methods 2.2.4). The overall values are lower in T1D boys compared to controls throughout the time studied. The maximum value in the T1D boys of 312ng/ml is achieved at approximately 0.9 years after PHV compared to 393ng/ml in the control boys at 1.25 years after PHV (Figure 5.3b). Thereafter the levels decrease in the T1D boys more rapidly than in the control boys (see also Table 5.3a).

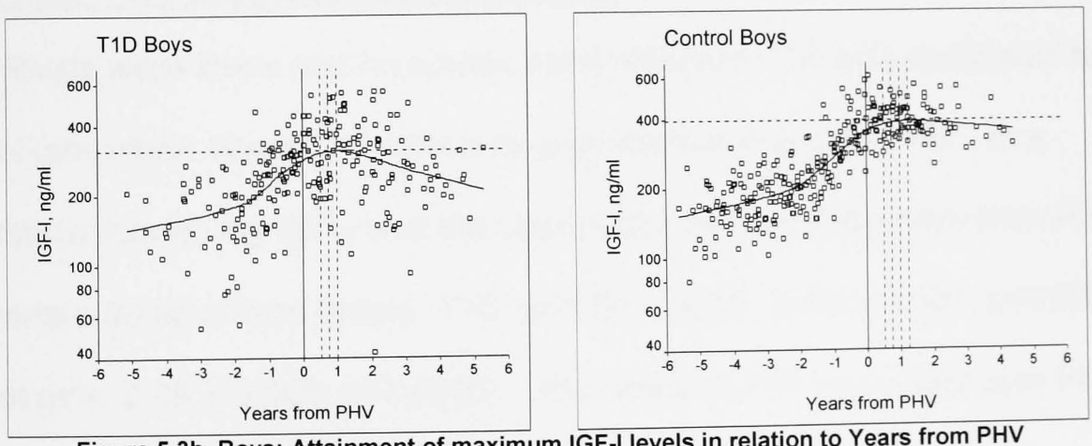


Figure 5.3b, Boys: Attainment of maximum IGF-I levels in relation to Years from PHV

5.3.1.4 IGF-I in relation to Puberty Stages

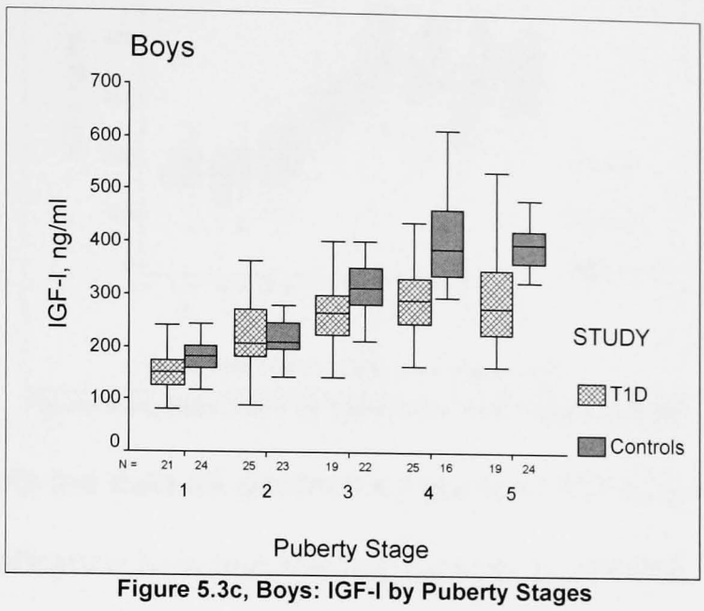


Figure 5.3c, Boys: IGF-I by Puberty Stages

IGF-I levels were lower in T1D boys compared to control boys in all puberty stages. This was significant in stage 1 ($p=0.003$), stage 3 ($p=0.02$), stages 4 and 5 ($p<0.0005$).

5.3.2 Girls

5.3.2.1 IGF-I in relation to years from PHV

IGF-I levels were lower and increased more slowly in T1D girls compared to control girls when viewed in relation to years before and after PHV. In a covariance model, log IGF-I was the dependent variable with years from PHV as a covariate (id as a fixed factor): T1D girls (B ± SEM), 0.03 ± 0.007, $p<0.0005$; control girls, 0.06 ± 0.003, $p<0.0005$). The levels of IGF-I by whole year PHV groups are shown in Figure 5.3d for the two cohorts.

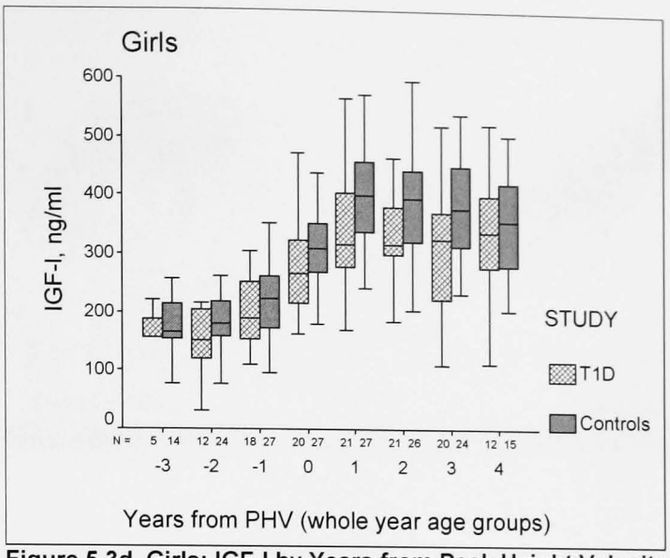


Figure 5.3d, Girls: IGF-I by Years from Peak Height Velocity

Table 5.3b presents the data as geometric means of IGF-I by whole years from PHV with the significance between the two cohorts presented.

Girls	-2	-1	0	1	2	3	4
T1D	140.37	191.68	268.58	315.81	329.71	245.12	254.54
Controls	181.39	213.16	311.43	396.22	377.93	300.51	268.59
P	NS	NS	.06	.009	NS	.02	NS

Table 5.3b, Girls: IGF-I levels by Years from Peak Height Velocity (Geometric Mean)

The T1D girls have lower levels of IGF-I at every time grouping around PHV and this is most statistically significant at one year after PHV.

5.3.2.2 Maximal Levels of IGF-I in relation to PHV

Loess was used to determine when and if a maximum level of IGF-I in relation to PHV was achieved. The maximum value in T1D girls compared to controls is lower although it is achieved at a similar time in relation to PHV. In the T1D girls, the maximum of 330ng/ml is achieved at approximately 1.5 years after PHV compared to 385ng/ml in the control girls at 1.6 years after PHV (Figure 5.3e) and both decline thereafter.

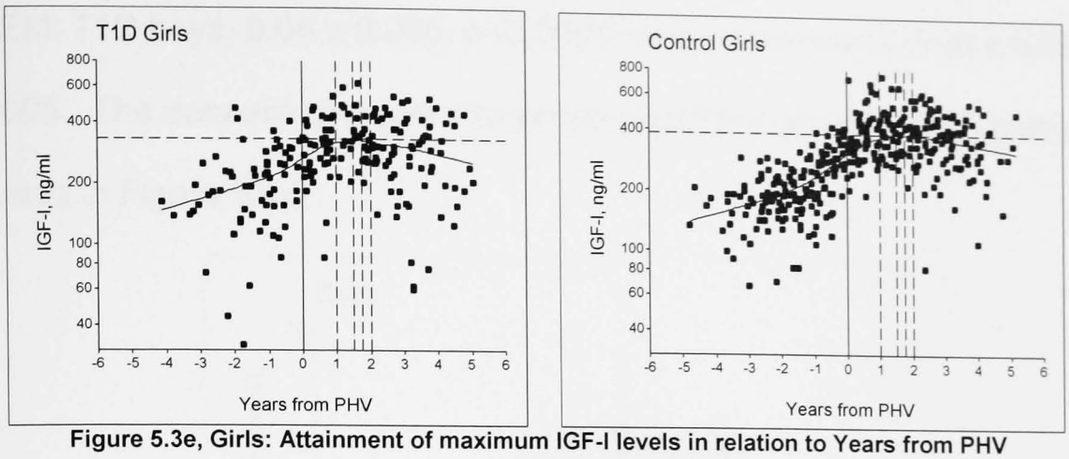


Figure 5.3e, Girls: Attainment of maximum IGF-I levels in relation to Years from PHV

5.3.2.4 IGF-I in relation to Puberty Stages

Interestingly, the girls with T1D did not have lower IGF-I levels compared to controls when grouped by puberty stages for stage 2 or 3 although they became significant at stage 4 (p=0.01) and remained so at stage 5 (p=0.04) (Figure 5.3f).

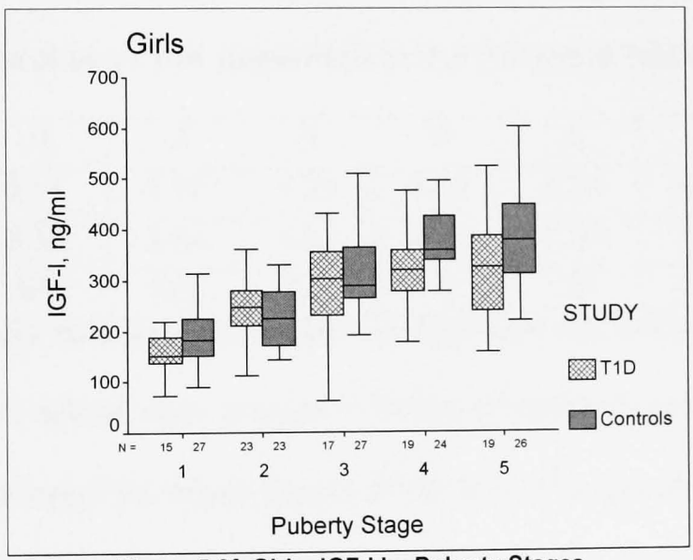


Figure 5.3f, Girls: IGF-I by Puberty Stages

5.4 Androstenedione (A4)

5.4.1 Boys

5.4.1.1 A4 in relation to years from PHV

Levels of A4 were lower and increased more slowly in T1D boys than controls in relation to years before and after PHV. In a covariance analysis with log A4 as the dependent variable, years from PHV as a covariate and id as a fixed factor,

B ± SEM: T1D boys, 0.04 ± 0.006, p<0.0005; and control boys, 0.08 ± 0.004, p<0.0005. The data are grouped into whole year PHV groups (± 0.5 year) and presented in Figure 5.4a.

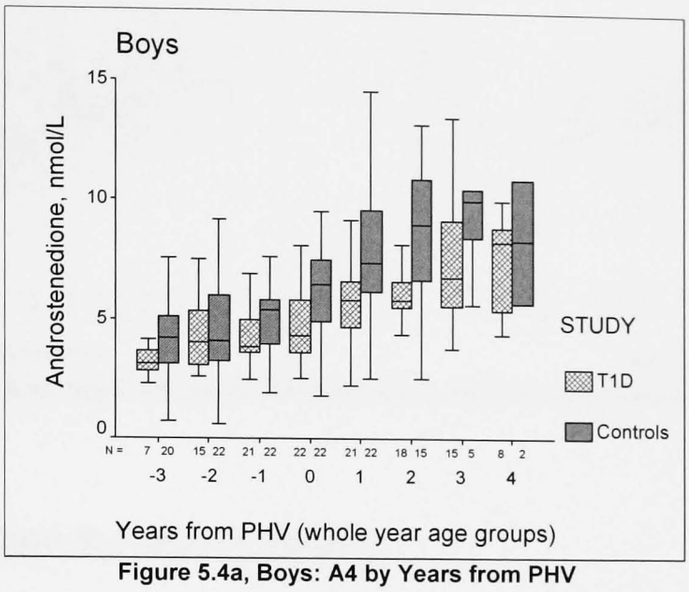


Figure 5.4a, Boys: A4 by Years from PHV

The geometric means of A4 by years from PHV and the significance between T1D boys and control boys are presented in the following table (Table 5.4a).

Boys	-3	-2	-1	0	1	2	3
T1D	3.11	4.12	4.28	4.31	5.34	6.16	7.01
Controls	3.59	3.64	4.63	5.63	7.00	8.02	9.25
P	NS	NS	NS	.04	.03	NS	NS

Table 5.4a, Boys: A4 levels nmol/L by Years from Peak Height Velocity (Geometric Mean)

Looking at the PHV whole year groups in terms of these geometric means, although there is a trend for lower levels of A4 in T1D boys, this only becomes statistically significant at PHV, p=0.04 and one year after PHV, p=0.03.

5.4.1.2 Maximal A4 levels in relation to PHV

Loess was again employed (Methods 2.2.4) to draw the line of best fit through the data. The maximal value of A4 achieved by the T1D boys (Figure 5.4b) is lower and later in relation to the timing of PHV than in the control boys: 8.1nmol/L at 5.3 years after PHV vs 10.3nmol/L at 4.3 years after PHV. On the

basis of this data, it is not possible to say whether this is a plateau or whether the values continue to increase in both cohorts.

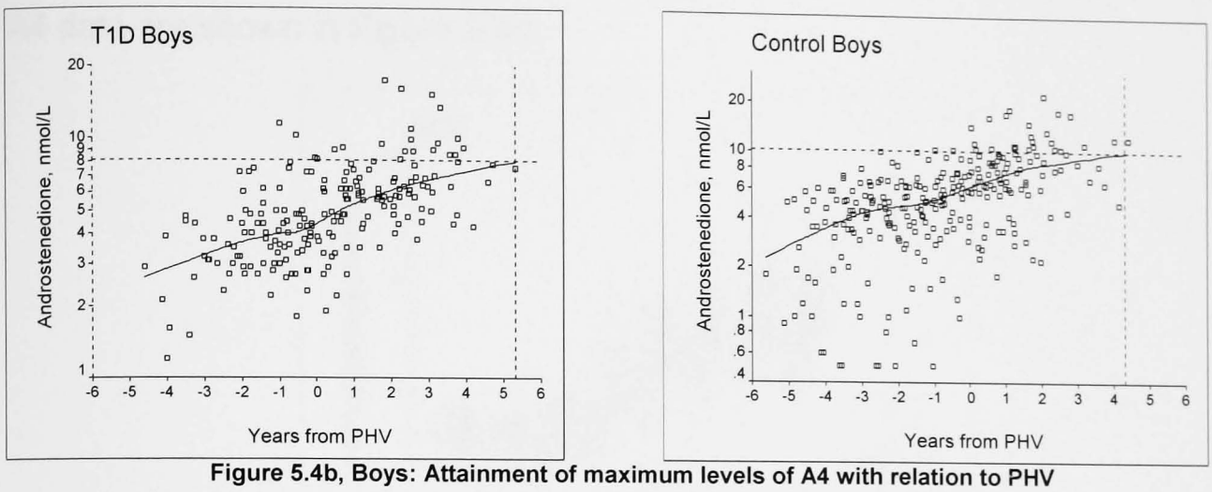


Figure 5.4b, Boys: Attainment of maximum levels of A4 with relation to PHV

5.4.1.4 A4 in relation to Puberty Stages

Puberty stage grouping of A4 levels in T1D and control boys shows little difference except at mid/late puberty (Figure 5.4c). A statistically significant difference (p=0.01) was observed at stage 4 between the two cohorts.

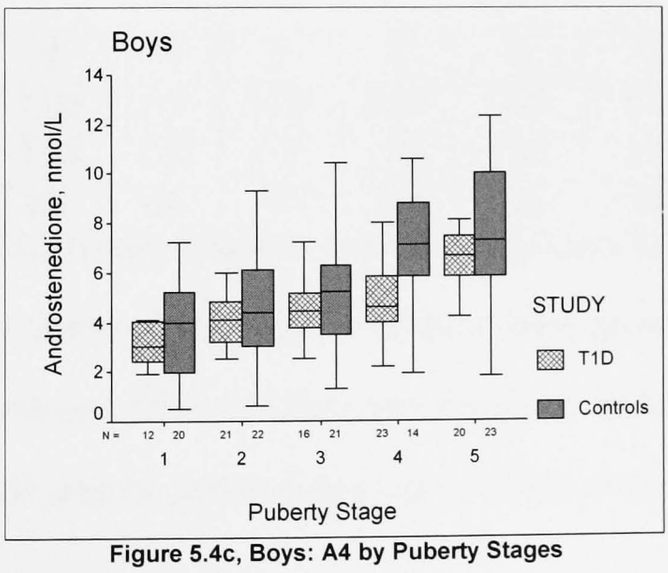


Figure 5.4c, Boys: A4 by Puberty Stages

5.4.2 Girls

5.4.2.1 A4 in relation to years from PHV

Levels of A4 were lower and increased more slowly in T1D girls than controls in relation to years before and after PHV. In a covariance analysis with log A4 as the dependent variable, years from PHV as a covariate and id as a fixed factor,

B ± SEM: T1D girls, 0.07 ± 0.008, p<0.0005; and control girls, 0.08 ± 0.004, p<0.0005. To compare the two cohorts, whole year age groups (± 0.5 years) of A4 data are shown in Figure 5.4d.

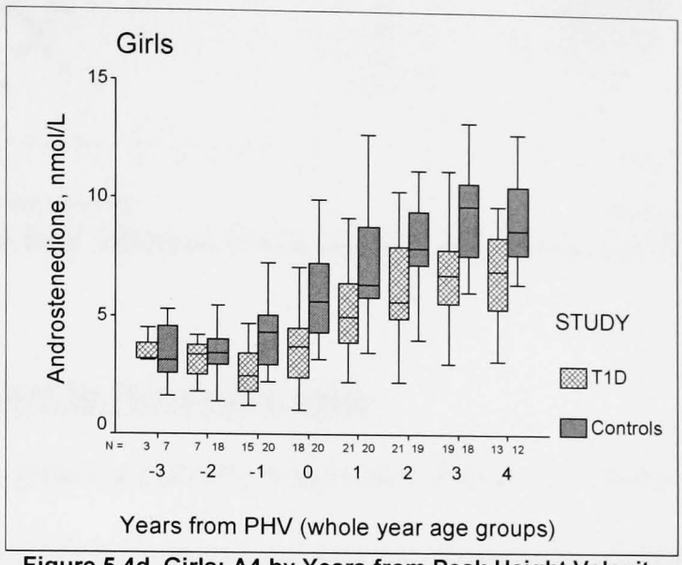


Figure 5.4d, Girls: A4 by Years from Peak Height Velocity

The geometric means of A4 by whole year age groupings from PHV and the significance between T1D girls and control girls are presented in the following table (Table 5.4b).

Girls	-2	-1	0	1	2	3	4
T1D	2.89	2.37	3.26	4.94	5.59	6.27	6.29
Controls	3.46	4.00	5.71	6.75	7.97	9.21	8.97
P	NS	.001	.001	.007	.006	<.0005	.009

Table 5.4b, Girls: A4 levels by Years from PHV (Geometric Mean)

Looking at the PHV whole year groups in terms of these geometric means, there is a statistically significant difference between the two cohorts at one year before PHV, p=0.001 and for all the subsequent time periods studied.

5.4.2.3 Maximal A4 levels in relation to PHV

Loess was again employed (Methods 2.2.4) to draw the line of best fit through the data. The maximal value of A4 achieved by the T1D girls (Figure 5.4e) is lower than in the control girls although the timing is similar: 7.7nmol/L at 5.1 years after PHV vs 10 nmol/L at 5.2 years after PHV.

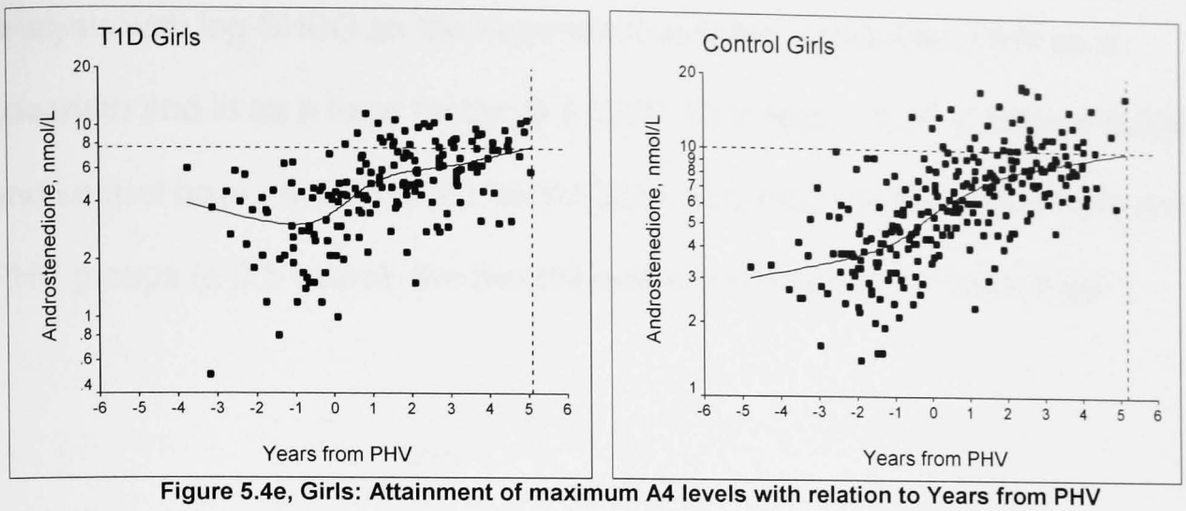


Figure 5.4e, Girls: Attainment of maximum A4 levels with relation to Years from PHV

5.4.2.4 A4 in relation to Puberty Stages

A4 analyses in the girls by puberty stage are shown in Figure 5.4f. Levels of A4 tend to be lower in T1D girls than controls for all puberty stages except stage 4. The difference is highly statistically significant at stage 3, p=0.008 and stage 5, p=0.002; and nearly significant at stage 2, p=0.06.

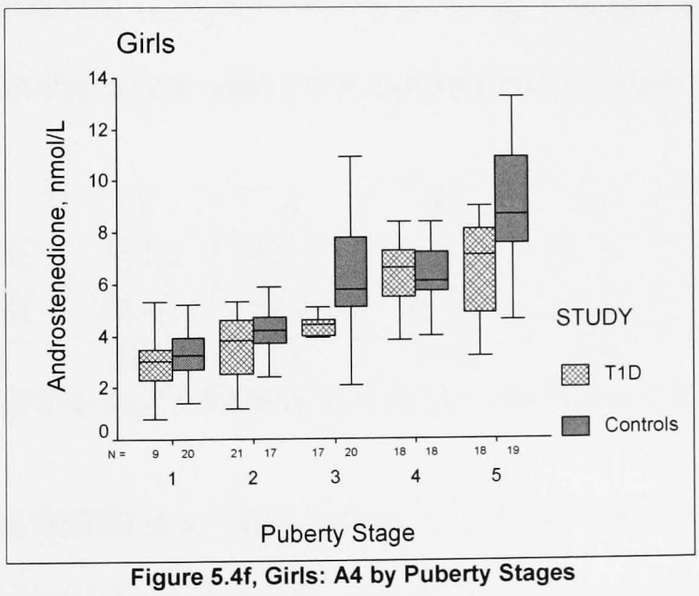


Figure 5.4f, Girls: A4 by Puberty Stages

5.5 SHBG

5.5.1 Boys

5.5.1.1 SHBG in relation to years from PHV

Levels of SHBG decrease from 3 years before PHV to 3 years after PHV in both control and T1D boys and were higher in T1D boys throughout. In a covariance

analysis with log SHBG as the dependent variable, years from PHV as a covariate and id as a fixed factor; B ± SEM: T1D boys, - 0.06 ± .004, p<0.0005; and control boys - 0.09 ± 0.003, p<0.0005. Grouping the data into whole year PHV groups (± 0.5 years), the two cohorts are compared in Figure 5.5a.

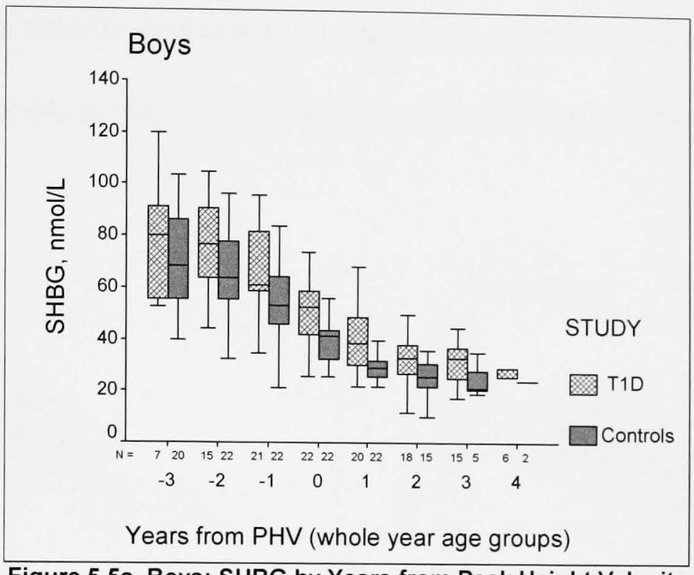


Figure 5.5a, Boys: SHBG by Years from Peak Height Velocity

To quantify the difference between the two cohorts, the data are presented as geometric means in the whole year PHV age groups in Table 5.5a.

Boys	-3	-2	-1	0	1	2	3
T1D	74.44	73.84	65.78	49.34	38.34	32.45	30.96
Controls	66.48	63.97	53.66	36.45	27.97	25.10	24.21
P	NS	NS	.02	.004	.004	.05	NS

Table 5.5a, Boys: SHBG levels by Years from PHV (Geometric Mean)

The mean levels of SHBG are higher in the T1D boys from 3 years before to 3 years after PHV and this is statistically significant 1 year before PHV and remains so until 2 years after PHV. The data are insufficient thereafter to comment on.

5.5.1.2 Maximal SHBG levels in relation to PHV

The Loess SPSS graphical procedure of fitting a best line through the individual points has been applied to the SHBG data (Figure 5.5b). The shape of these

graphs in the 2 cohorts of boys is similar and appears as an inverted elongated "S". In the T1D boys; at 1.75 years before PHV, when SHBG is near 73 nmol/L, the decline appears to accelerate and decreases until 1 year after PHV occurs. In the control boys, this period of decrease begins at 1.5 years before PHV when SHBG is at a lower level of 58nmol/L and proceeds until 1 year after PHV. In both groups this rate of decrease starts to slow down approximately 1 year after PHV and subsequently continues to slowly decrease (perhaps more slowly in T1D).

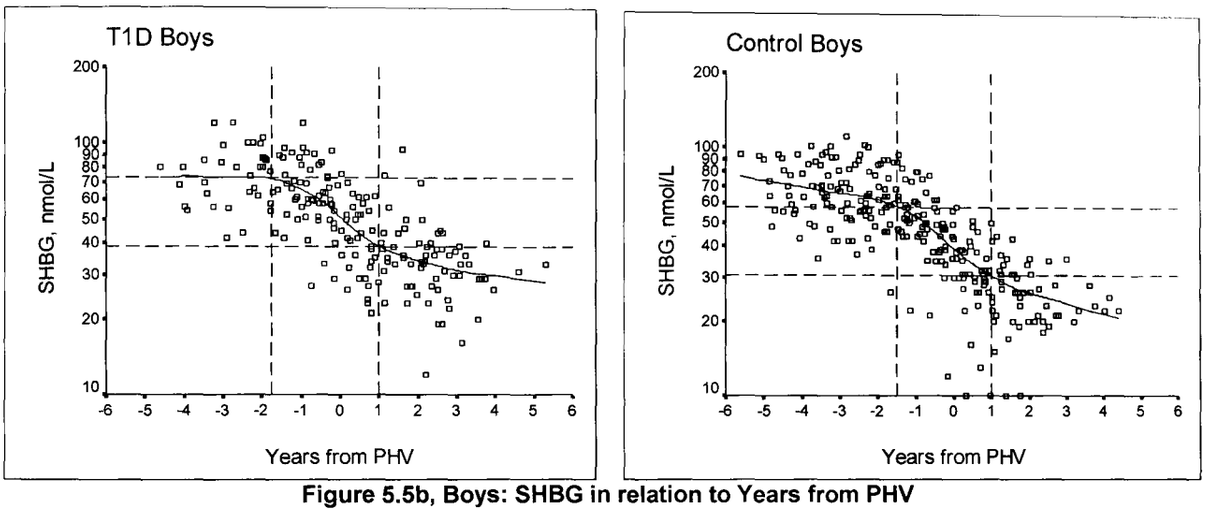


Figure 5.5b, Boys: SHBG in relation to Years from PHV

5.5.1.3 SHBG in relation to Puberty Stages

SHBG levels are higher in each puberty stage for the T1D boys compared to the control boys. This difference is statistically significant at stage 3, p=0.04 and stage 5, p =0.01.

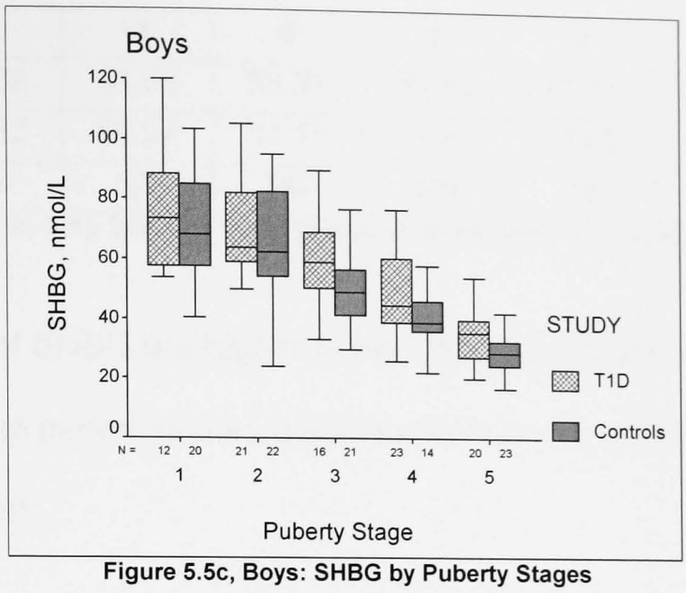


Figure 5.5c, Boys: SHBG by Puberty Stages

5.5.2 Girls

5.5.2.1 SHBG in relation to years from PHV

SHBG levels decrease from 2 years before PHV to 4 years after in both cohorts of girls. In a covariance analysis with log SHBG as the dependent variable, years from PHV as a covariate and id as a fixed factor; B ± SEM: T1D girls, -0.01 ± .005, p=0.008; and control girls, -0.04 ± .003, p<0.0005. Grouping the data into whole year age groups (± 0.5 years), the two cohorts are compared in Figure 5.5d.

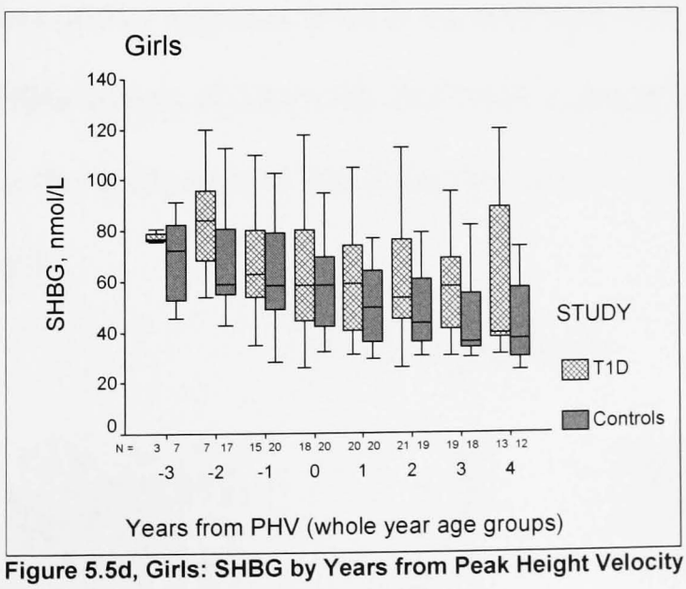


Figure 5.5d, Girls: SHBG by Years from Peak Height Velocity

Table 5.5b presents the data as geometric means of SHBG by whole years from PHV with the significance of the difference between the two cohorts presented.

Girls	-2	-1	0	1	2	3	4
T1D	80.82	64.00	58.32	55.80	57.81	54.85	54.72
Controls	65.83	59.24	53.17	47.47	46.26	42.00	42.49
P	NS	NS	NS	NS	.06	.02	NS

Table 5.5b, Girls: SHBG by Years from Peak Height Velocity (Geometric mean)

The mean levels of SHBG are higher in the T1D girls compared to control girls throughout the time period studied but this only reaches statistical significance at 3 years after PHV.

5.5.2.2 Maximal SHBG levels in relation to PHV

A 'best fit' line through the individual points using the Loess method of SPSS has been applied to the SHBG data (Figure 5.5e). The shape of these graphs is different in the two cohorts of girls. The first phase of the graph in the T1D girls is a decreasing straight line that starts in the years before PHV until 0.6 years after PHV when it then appears to plateau until 3.2 years after PHV and then continues a slow decline. At 2.5 years before PHV, SHBG is 78nmol/L and at 0.6 years after PHV it has reached 55nmol/L. The picture in the control girls is the opposite, the first phase appears to be a steady state until 1.65 years before PHV with mean SHBG values of 63nmol/L and then a straight line decline begins that lasts for the study period and if we look until 4 years after PHV when levels are 37.5nmol/L.

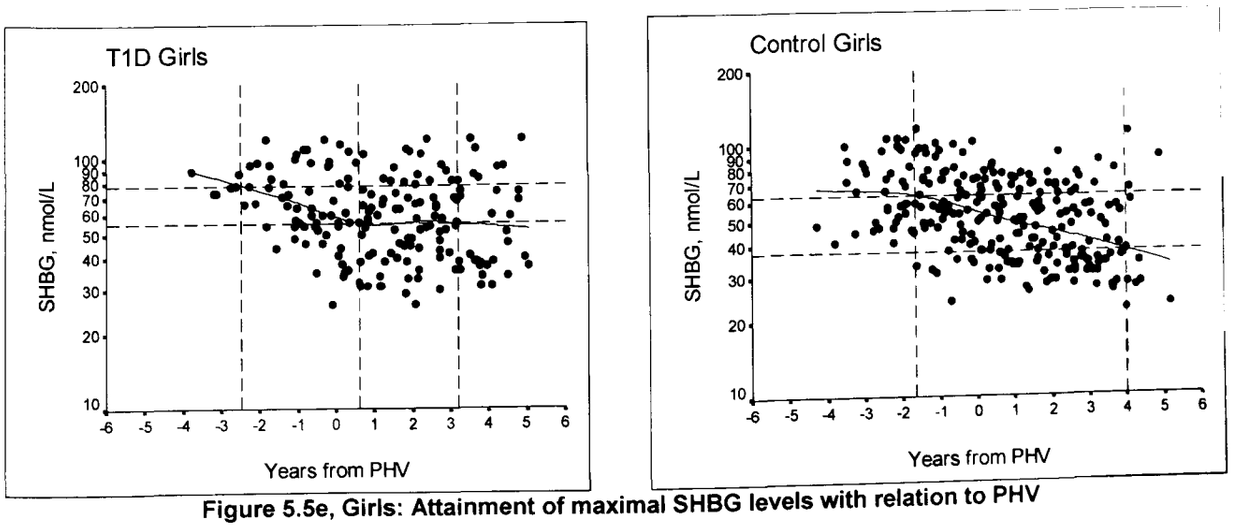


Figure 5.5e, Girls: Attainment of maximal SHBG levels with relation to PHV

5.5.2.3 SHBG in relation to Puberty Stages

Levels of SHBG grouped by puberty stages for both cohorts of girls are shown in Figure 5.5f. Although SHBG levels are similar or greater in both groups of girls, there is no statistically significant difference between the two cohorts SHBG levels at any puberty stage.

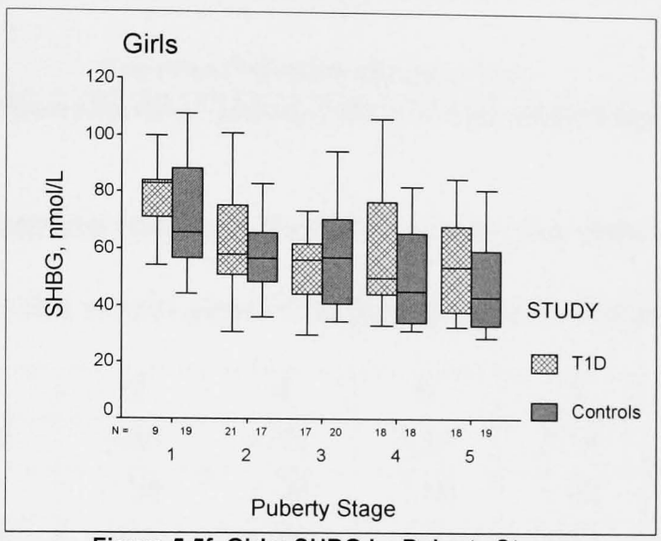


Figure 5.5f, Girls: SHBG by Puberty Stages

5.6 Leptin

5.6.1 Boys

5.6.1.1 Leptin in relation to years from PHV

Levels of leptin were markedly higher in T1D boys throughout the study period and decreased from 1 year before PHV to 3 years after PHV in both control and T1D boys. In a covariance analysis with log leptin as the dependent variable, years from PHV as a covariate and id as a fixed factor; B ± SEM: T1D boys, -0.02 ± .006, $p<0.0005$; and control boys – 0.03 ± 0.004, $p<0.0005$. Grouping the data into whole year PHV groups (± 0.5 years); the two cohorts are compared in Figure 5.6a.

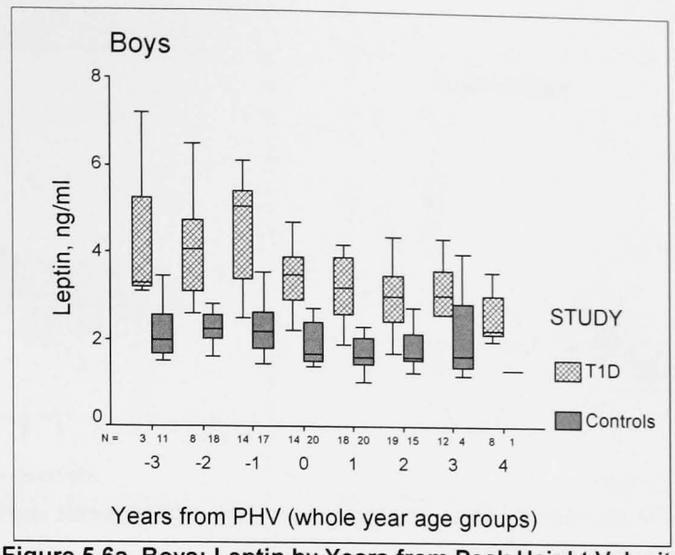


Figure 5.6a, Boys: Leptin by Years from Peak Height Velocity

To quantify the difference between the two cohorts, the data are presented as geometric means in the whole year PHV age groups in Table 5.6a.

Boys	-3	-2	-1	0	1	2	3
T1D	4.19	3.95	4.73	3.38	3.14	2.97	3.13
Controls	2.11	2.38	2.38	2.06	1.92	1.91	1.88
P	.006	.001	<0.0005	.001	.002	.001	NS

Table 5.6a, Boys: Leptin by Years from Peak Height Velocity (Geometric mean)

Leptin levels are significantly higher in T1D boys throughout the whole study period.

5.6.1.2 Maximal Leptin levels in relation to PHV

The Loess graphical method (SPSS) was used to explore if and at what time the curve of leptin reached a maximum/plateau in relation to PHV (Methods 2.2.4). In the T1D boys, leptin levels appear to increase to a maximum of 4.2 ng/ml at 1.5 years before PHV and then slowly decrease and plateau at 2.9 ng/ml at 3 years after PHV. There does not appear to be an increase in the control boys before PHV but rather a plateau at 2.2ng/ml from the start of the study period until 1.4 years before PHV and then a decline to 1.6ng/ml which is maintained from 1 to 1.6 years after PHV and which then may slowly decline although the data at this point is sparse.

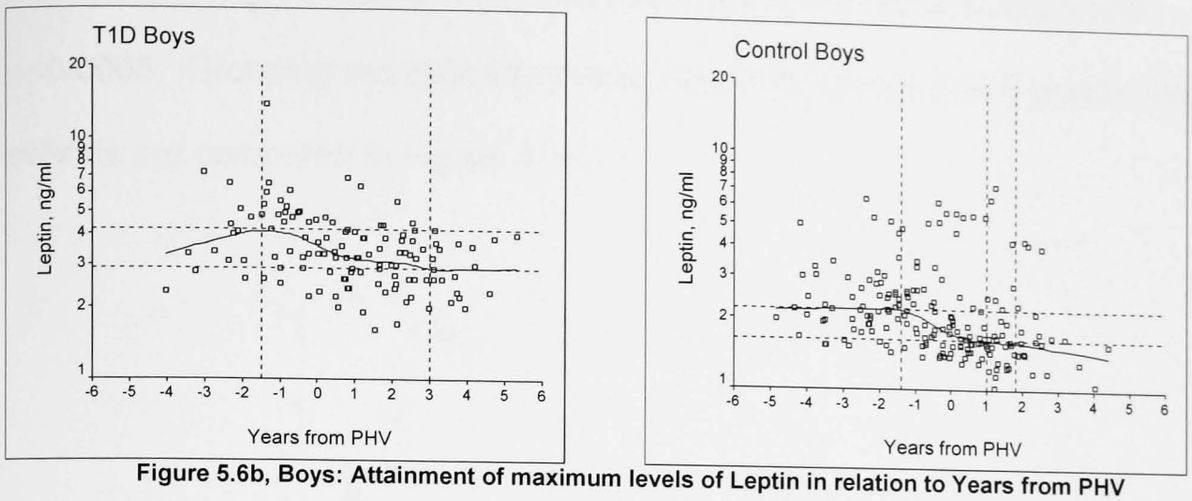


Figure 5.6b, Boys: Attainment of maximum levels of Leptin in relation to Years from PHV

5.6.1.4 Leptin in relation to Puberty Stages

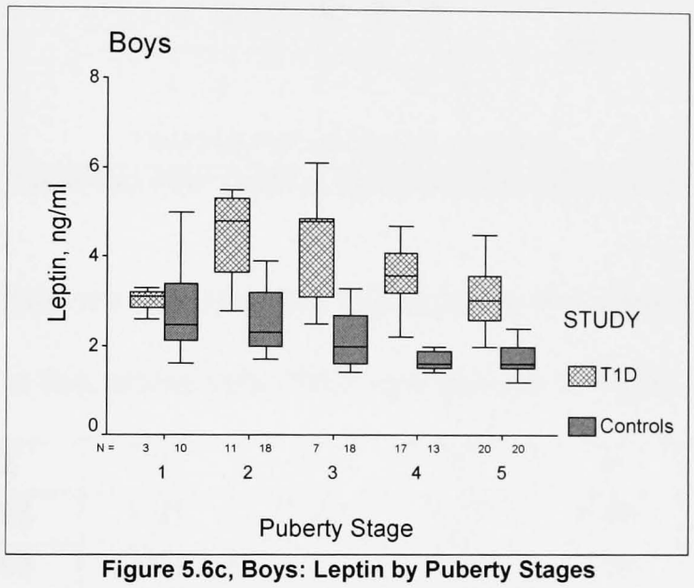


Figure 5.6c, Boys: Leptin by Puberty Stages

Leptin levels were higher in T1D boys compared to control boys in all puberty stages. This was statistically significant in stages 2, 3, 4 and 5 where the p values were <0.0005, 0.007, <0.0005 and <0.0005 respectively.

5.6.2 Girls

5.6.2.1 Leptin in relation to years from PHV

Levels of leptin in the girls were again markedly higher in T1D cohort throughout the study period. Levels increased from 2 years before PHV to 4 years after PHV in both control and T1D girls. In a covariance analysis with log leptin as the dependent variable, years from PHV as a covariate and id as a fixed factor;

B ± SEM: T1D girls, 0.08 ± .009, p<0.0005; and control girls 0.06 ± 0.005, p<0.0005. Grouping the data into whole year PHV groups (± 0.5 years); the two cohorts are compared in Figure 5.6d.

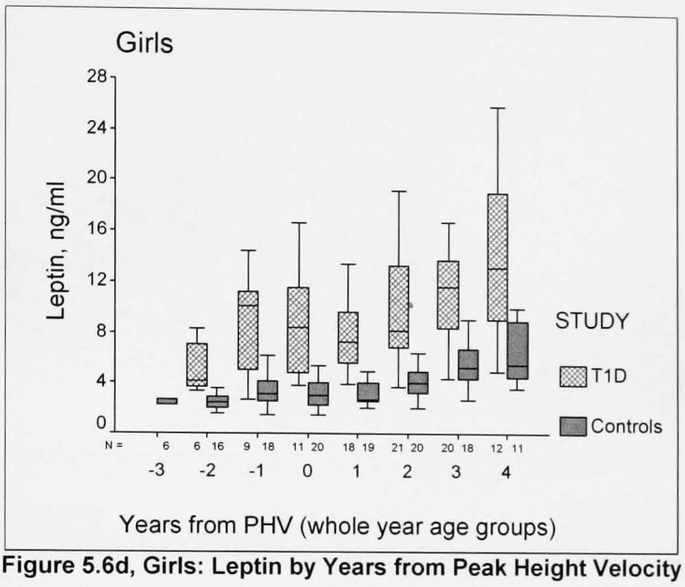


Figure 5.6d, Girls: Leptin by Years from Peak Height Velocity

To quantify the difference between the two cohorts, the data are presented as geometric means in the whole year PHV age groups in Table 5.6b.

Girls	-2	-1	0	1	2	3	4
T1D	4.85	7.61	7.50	7.50	8.96	10.71	12.37
Controls	2.48	3.14	3.07	3.22	3.96	5.51	6.01
P	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	.002

Table 5.6b, Girls: Leptin by Years from Peak Height Velocity (Geometric mean)

Leptin levels are significantly higher in T1D girls throughout the whole study period.

5.6.2.2 Maximal Leptin levels in relation to PHV

Loess was again used to draw the line of best fit through the leptin data in relation to PHV (Methods 2.2.4). In the T1D girls, there is a continual almost straight line increase throughout the study period reaching a maximum of 12.75ng/ml at 4.75 years after PHV. The data does not extend beyond that point and it is therefore not possible to tell if leptin levels then plateau or

continue to increase. In the control girls there is an increase from 3/4 years before PHV to 0.4 years before where the level plateaus at 2.95ng/ml until 0.55 years after PHV. At this point it appears to increase again to 7ng/ml at 4.85 years after PHV where the data ends so again it is not possible to know what happens thereafter.

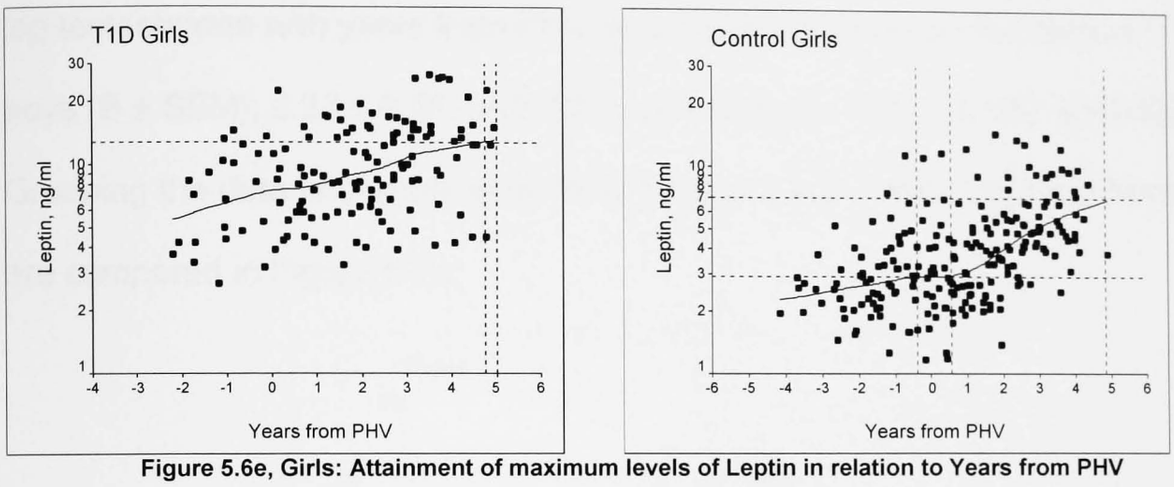


Figure 5.6e, Girls: Attainment of maximum levels of Leptin in relation to Years from PHV

5.6.2.3 Leptin in relation to Puberty Stages

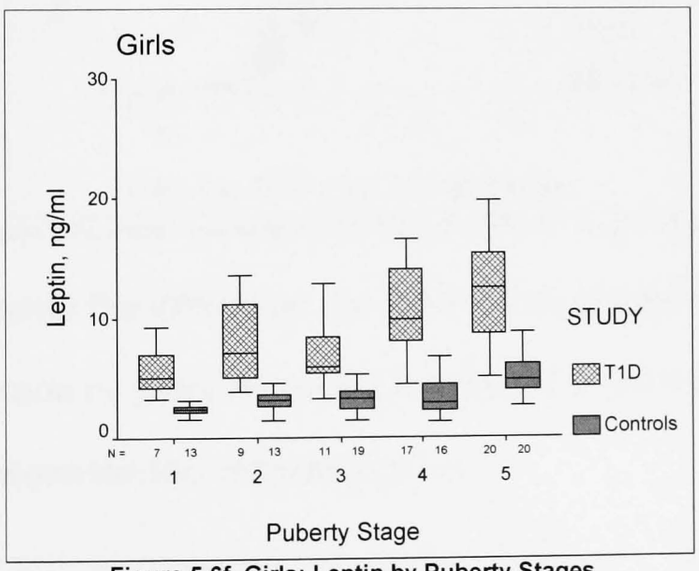


Figure 5.6f, Girls: Leptin by Puberty Stages

Leptin levels were higher in T1D girls compared to control girls in all puberty stages. This was statistically significant at a $p < 0.0005$ for stages 1-5.

5.7 Testosterone

5.7.1 Boys

5.7.1.1 Testosterone in relation to years from PHV

Testosterone levels were lower and increased more slowly in T1D boys compared to control boys. As the dependent variable in a covariance model, log testosterone with years from PHV as a covariate (id as a fixed factor): T1D boys (B ± SEM), 0.27 ± 0.01, p<0.0005; control boys, 0.33 ± 0.008, p<0.0005). Grouping the data into whole year PHV groups (± 0.5 years); the two cohorts are compared in Figure 5.7a.

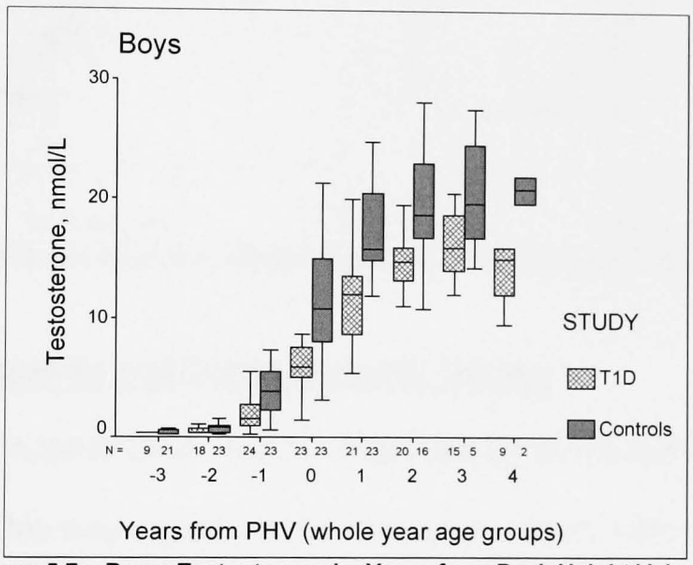


Figure 5.7a, Boys: Testosterone by Years from Peak Height Velocity

To statistically quantify the difference, the data are presented as geometric means of testosterone by years from PHV in table 5.7a and the significance of the difference between the two cohorts is given.

Boys	-3	-2	-1	0	1	2	3
TID	0.43	0.52	1.43	5.44	10.95	14.05	16.72
Controls	0.39	0.66	3.12	10.23	16.75	19.06	19.97
P	NS	NS	.001	<0.0005	<0.0005	.009	NS

Table 5.7a, Boys: Testosterone by Years from Peak Height Velocity (Geometric mean)

The difference in testosterone levels is statistically significant from one year before to 2 years after PHV.

5.7.1.2 Maximal Testosterone levels in relation to PHV

The line of best fit was again drawn by loess (SPSS) through the testosterone data in relation to years from PHV. An 'S' shaped curve (the mirror image of the SHBG curves) was obtained for both cohorts with a maximum level of 16nmol/L of testosterone reached at 4 years after PHV in the T1D boys and 26nmol/L in the control boys marginally later at 4.3 years post PHV.

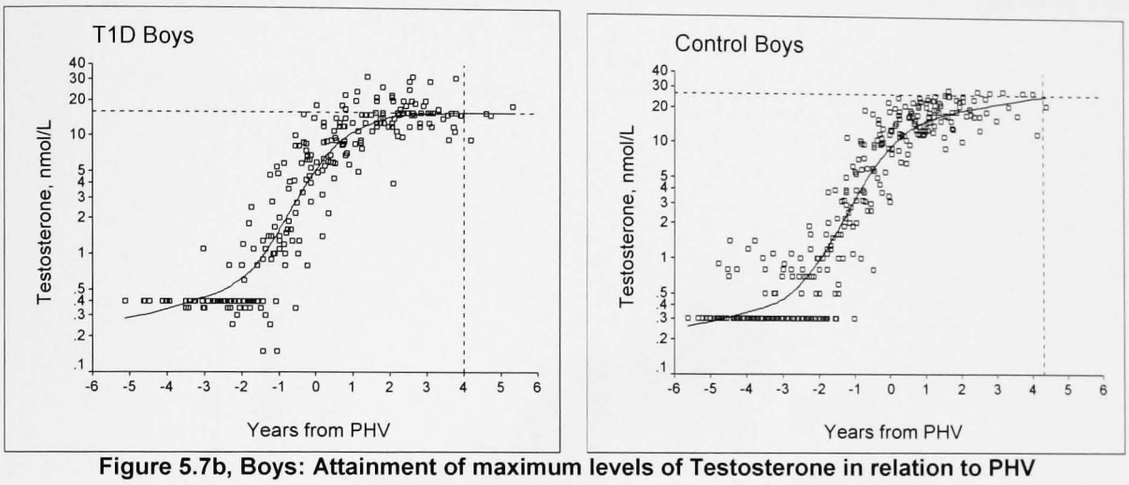


Figure 5.7b, Boys: Attainment of maximum levels of Testosterone in relation to PHV

5.7.1.4 Testosterone in relation to Puberty Stages

Testosterone levels were lower in T1D boys compared to control boys in all puberty stages. This was significant in all stages except stage 2; stage 1 ($p=0.03$), stage 3 ($p=0.003$), stages 4 ($p=0.01$) and 5 ($p<0.02$).

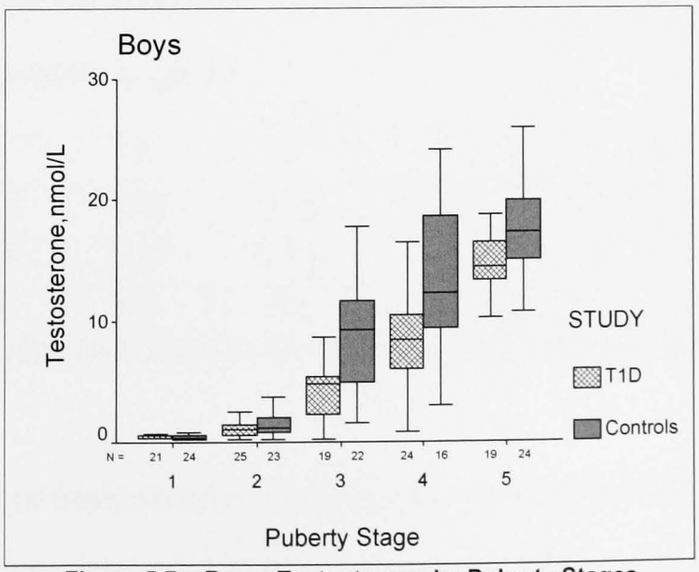


Figure 5.7c, Boys: Testosterone by Puberty Stages

5.7.2 Girls

5.7.2.1 Testosterone in relation to years from PHV

Testosterone levels were lower in T1D girls compared to control girls. As the dependent variable in a covariance model, log testosterone with years from PHV as a covariate (id as a fixed factor): T1D girls (B ± SEM), 0.06 ± 0.01, $p<0.0005$; control girls, 0.11 ± 0.005, $p<0.0005$). Grouping the data into whole year PHV groups (± 0.5 years); the two cohorts are compared in Figure 5.7d.

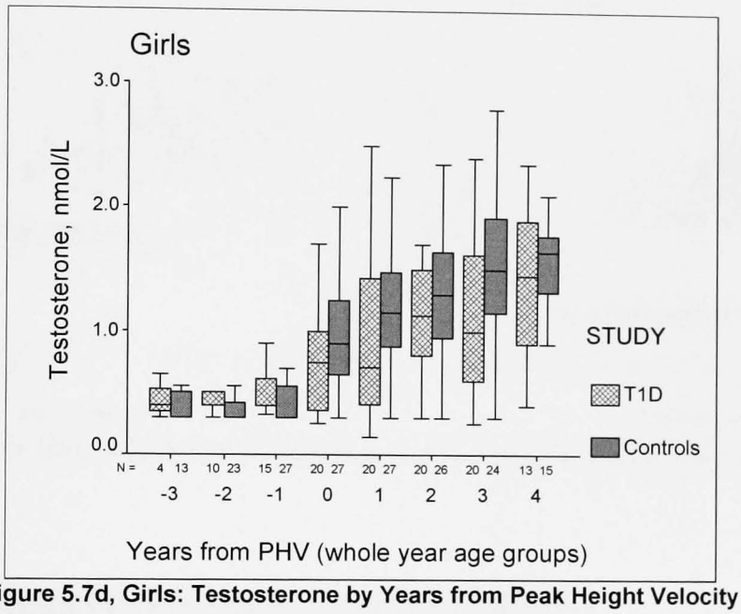


Figure 5.7d, Girls: Testosterone by Years from Peak Height Velocity

The data are presented as geometric means of testosterone by years from PHV in table 5.7b and the significance of the difference (based on logged data) between the two cohorts is given.

Girls	-2	-1	0	1	2	3	4
TID	0.45	0.48	0.65	0.69	0.99	0.96	1.21
Controls	0.38	0.43	0.76	1.05	1.14	1.35	1.54
P	NS	NS	NS	.03	NS	.06	NS

Table 5.7b, Girls: Testosterone by Years from Peak Height Velocity (Geometric mean)

Although the level of testosterone tends to be lower in the T1D girls from the time of PHV onwards, it is only statistically significant ($p=0.03$) at one year after PHV.

5.7.2.2 Maximal Testosterone levels in relation to PHV

The line of best fit through the testosterone data in relation to years from PHV was again drawn by loess (SPSS). The shape of the curve for the T1D girls is a rather flattened 'S'shape while that of the control girls appears properly 'S' shaped. The curve of the T1D girls reaches a maximum of 1.3nmol/L at 5.1 years after PHV and that of the controls is 1.7nmol/L at nearly the same time, 5.2 years after PHV.

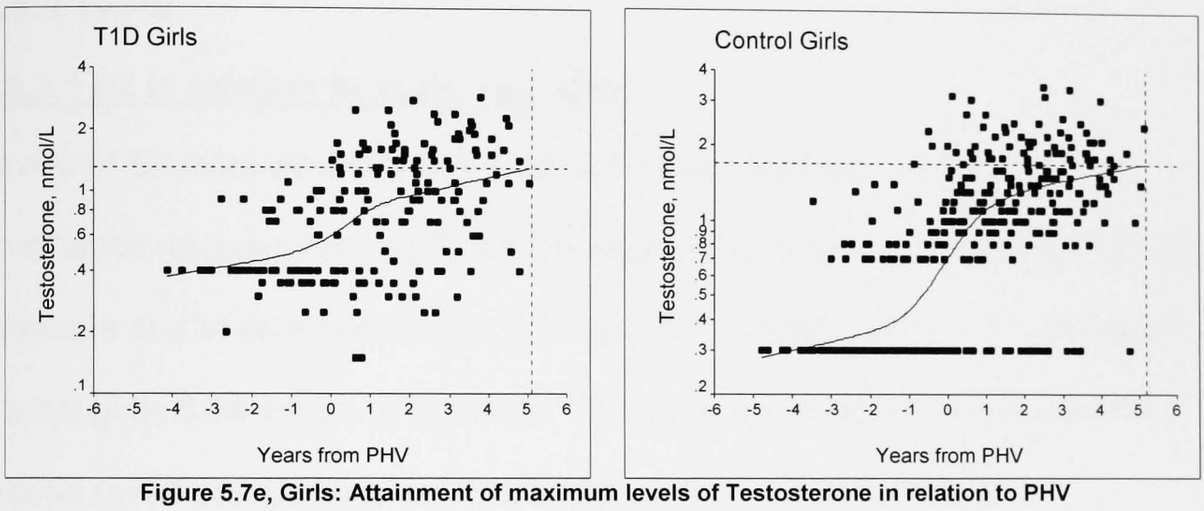


Figure 5.7e, Girls: Attainment of maximum levels of Testosterone in relation to PHV

5.7.2.3 Testosterone in relation to Puberty Stages

T1D girls had significantly higher testosterone levels than controls at puberty stage 1 (p = 0.03) and significantly lower levels at stage 5 (p = 0.05).

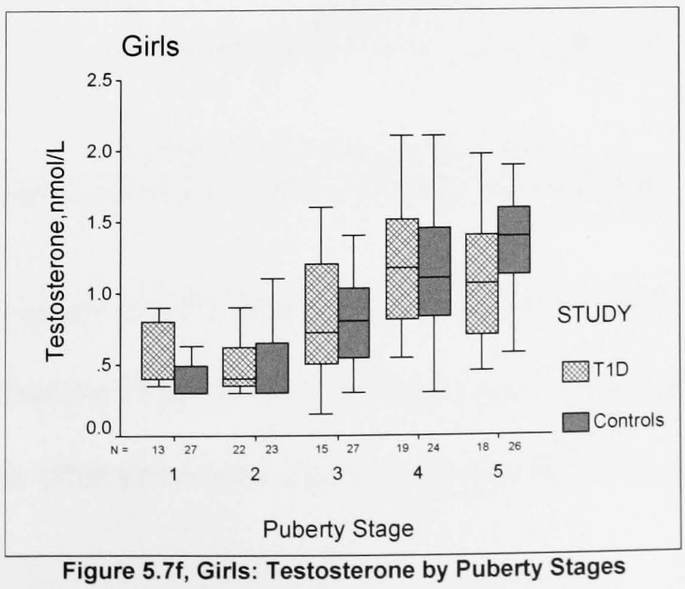


Figure 5.7f, Girls: Testosterone by Puberty Stages

5.8 Oestradiol (E2)

5.8.1 Boys

Oestradiol was not measured in the control boys and so there is no comparison.

The geometric means for the T1D boys are given for information.

Boys	-3	-2	-1	0	1	2	3
TID	33.32	26.05	25.25	30.60	36.69	45.09	83.43
Controls	Not done						

Table 5.8a, Boys: Oestradiol by Years from Peak Height Velocity (Geometric mean)

5.8.2 Girls

5.8.2.1 E2 in relation to years from PHV

Levels of E2 increased slowly in both cohorts in relation to PHV. In a covariance analysis with log E2 as the dependent variable, years from PHV as a covariate and id as a fixed factor, T1D girls (B ± SEM), 0.17 ± 0.01, $p<0.0005$; control girls, 0.24 ± 0.01, $p<0.0005$). Grouping the data into whole year PHV groups (± 0.5 years); the two cohorts are compared in Figure 5.8a.

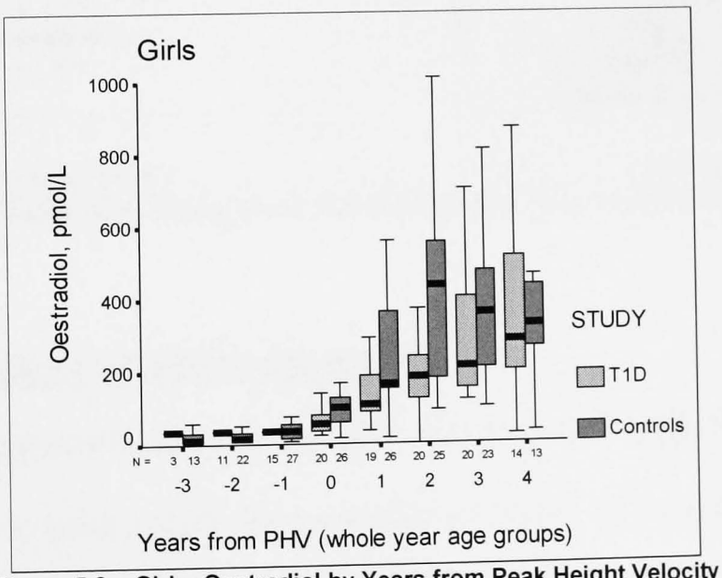


Figure 5.8a, Girls: Oestradiol by Years from Peak Height Velocity

Levels of E2 were higher in T1D girls before PHV although this was only significant 2 years before PHV, once PHV had occurred levels were higher in control girls and this difference was statistically significant 2 years after PHV.

Girls	-2	-1	0	1	2	3	4
T1D	38.14	36.97	50.48	117.41	158.46	245.12	254.54
Controls	18.72	33.14	74.83	175.08	302.08	300.51	268.59
P*	<0.0005	NS	.06	.07	.03	NS	NS

Table 5.8b, Girls: Oestradiol by Years from Peak Height Velocity (Geometric mean)

5.8.2.2 Maximal E2 levels in relation to PHV

The best-fit line was again drawn through the data by loess (SPSS) for both cohorts. The girls with T1D appear to have higher levels of E2 three years before PHV and their 'S' shaped increase reaches a maximum of 240pmol/L at 4.7 years after PHV. The control girls start from a lower E2 level at 1-3 years before PHV and have a steeper rise to 280 pmol/L at 2.4 years after PHV.

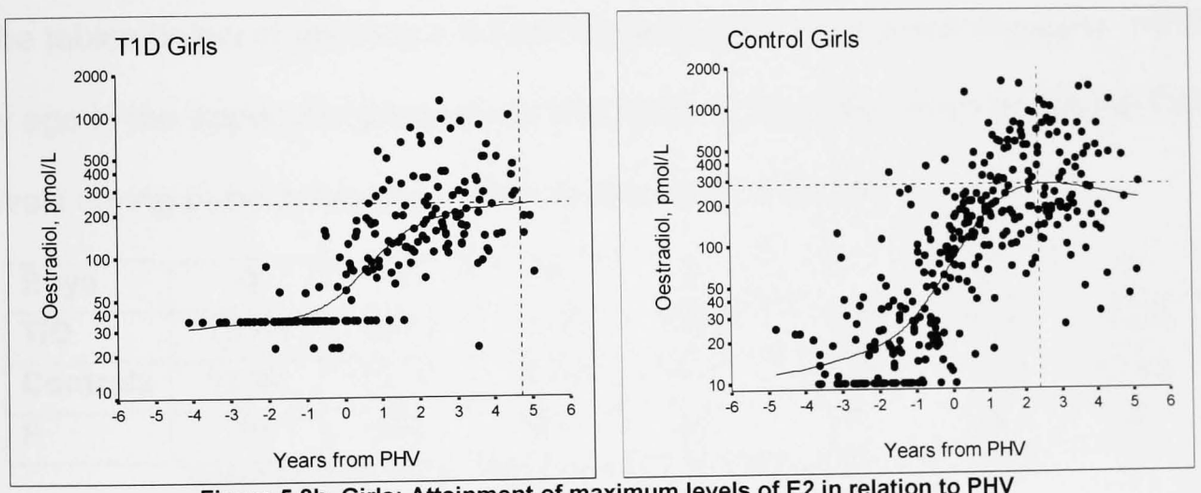


Figure 5.8b, Girls: Attainment of maximum levels of E2 in relation to PHV

5.8.2.4 E2 in relation to Puberty Stages

E2 comparison by puberty stage in the girls is shown in Figure 5.8c. E2 levels tend to be higher in control girls compared to girls with T1D at all puberty stages from 2 to 5 although the difference is not statistically significant. The T1D girls, however, have significantly higher (p= 0.004) E2 levels prepubertally (stage 1) than the control girls.

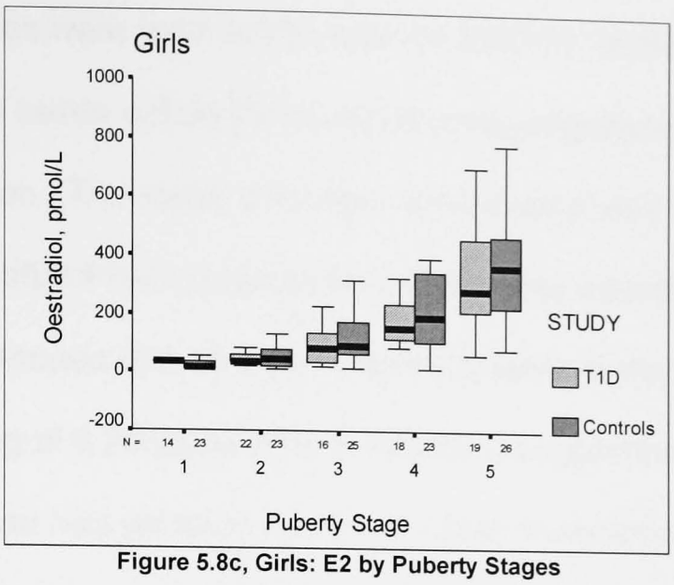


Figure 5.8c, Girls: E2 by Puberty Stages

5.9 Free thyroxine (FT4)

The tables below of geometric means by years from PHV and the graphs of FT4 by age in the appendix demonstrate that there is very little difference in the FT4 levels during puberty between the two cohorts of children.

Boys	-3	-2	-1	0	1	2	3
TID	13.76	14.71	14.52	13.59	13.26	14.52	15.86
Controls	15.93	15.71	14.99	14.01	15.10	15.68	14.53
P	.05	NS	NS	NS	.07	NS	NS

Table 5.9a, Boys: FT4 by Years from Peak Height Velocity (Geometric Mean)

Girls	-2	-1	0	1	2	3	4
TID	14.43	13.91	14.18	12.74	13.37	14.54	11.95
Controls	15.29	14.08	13.17	13.92	14.38	15.53	14.63
P	NS	NS	NS	NS	NS	NS	NS

Table 5.9b, Girls: FT4 by Years from Peak Height Velocity (Geometric Mean)

5.10 Summary and Discussion

A number of the hormones studied have lower values in the T1D children than the controls (DHEAS, IGF-I, A4, testosterone and oestradiol) whether examined in terms of peak height velocity or puberty.

Dramatic differences were seen in this data for DHEAS, which was lower in the T1D cohort in both sexes before PHV and became progressively more so with pubertal progression. The mean maximum values were very different and were not achieved until after PHV in both sexes. There was a tempo difference in the girls; T1D girls plateaued at 0.75 years after PHV while in the control girls, levels were still increasing at 5.25.years after PHV with a suggestion (but insufficient data) that no plateau had yet been reached. Other investigators have seen lower levels of DHEAS in T1D (Cohen et al. 1984; Loviselli et al. 1994; Radetti et al. 1994) although Small's study of 17 young men with T1D (mean age 21 years) found no difference in DHEAS levels in these subjects and age matched controls and no relationship of DHEAS and HbA1c (Small et al. 1989). Meyer et al found no difference in DHEAS levels in either sex between T1D subjects and puberty matched controls. Their cohort of T1D adolescents appears to be exceedingly well controlled with an insulin dose that did not increase significantly during puberty and at stage 5 was 0.98 ± 0.26 U/kg/d in males and 0.89 ± 0.08 U/kg/d in females. In addition there was no significant difference in HbA1c levels between puberty groups and at stage 5 they were 8.2 ± 1.4% and 8.4 ± 2.4% respectively in males and females (Meyer et al. 2000). Couch's study of 23 post pubertal T1D subjects observed that those designated as being in poor control (HbA1c>10%) had lower DHEAS levels than those in good control (HbA1c<8.0%) (Couch 1992). Ebeling et al explored the relationship of hyperinsulinaemia and DHEAS in T1D subjects using a 4-hour euglycaemic insulin clamp and found that DHEAS decreased with high levels of insulin. In 23 young men with 'good' control (mean HbA1c 7.8%) they observed that mean DHEAS concentration was in the normal range but that during an insulin infusion DHEAS levels decreased by 11% (Ebeling et al. 1995). Radetti's study

on 129 T1D children observed a negative and significant DHEAS-SDS (-0.36 ± 0.77) and a negative correlation of DHEAS-SDS and HbA1c although no relation between DHEAS and insulin dose. Citing the work of Kobayashi et al (Kobayashi et al. 1983) on the pharmacokinetics of insulin (which showed that supraphysiological insulin levels were achieved after each subcutaneous insulin injection) they claimed that every insulin administration would be expected to lower DHEAS regardless of the dose (Radetti et al. 1994).

Could we conjecture that in pubertal subjects with T1D there might be a synergistic effect of the insulin resistance of puberty as well as the multiple insulin injections that act in concert to suppress the levels of DHEAS in these subjects?

IGF-I was lower in the current T1D cohort compared to controls throughout the time period studied whether considered by years from PHV or by puberty stages. Lower levels in T1D children have almost universally been observed in numerous studies over many years (Amiel et al. 1984; Taylor et al. 1988; Rogers et al. 1991; Massa et al. 1993; Clayton et al. 1994; Radetti et al. 1997; Zachrisson et al. 1997; Cianfarani et al. 2000). While most of these studies have been cross sectional and have commented that levels peak at 'midpuberty' (Massa et al. 1993) or 'stage 4' (Clayton et al. 1994) our longitudinal study reinforces the finding that the peak of IGF-I occurs after PHV in both sexes in both control and T1D children. Although the levels rise throughout puberty in all the children, the growth velocity has started to slow while the levels of IGF-I continue to rise. The large study of Lofvist et al observed this by noting a positive relation between age and IGF-I in early puberty and then a negative association in late puberty (Lofqvist et al. 2001).

The maximum level in the boys occurred approximately 0.9 years after and 1.25 years after PHV in T1D and controls respectively. In terms of puberty stage this would appear to be stage 4 with little change in stage 5 in both groups. In the girls the time of the maximum level is very similar at 1.5 and 1.6 years after PHV, T1D and controls respectively. As in the boys, this looks like occurring in stage 4 with little change in stage 5.

On a purely speculative note, it would appear that the levels of IGF-I may overcome the abnormalities within the diabetic pubertal GH/IGF-I axis/insulin resistant pubertal state to enable growth in concert with the rise of GH and sex steroids at this challenging time. However, as the demands of the growth spurt diminish, there would appear to be a switch in priorities, such that the effects of IGF-I and the sex steroids concentrate more on body composition rather than growth, ie towards the development of muscle and adipose tissue as well as bone development.

To a certain extent the changes in total IGF-I presented here may not give us a true picture, as this does not reflect changes in IGF-I bioavailability. We know that IGF-I is modulated by the two binding proteins IGFBP-I (as an inhibitor) and BP-3 as the major carrier protein and without knowledge of their levels it is not possible to comment on the IGF-I bioavailability.

By contrast, leptin levels were strikingly higher in the T1D children compared to controls. In all boys, leptin levels decreased with time but whether in relation to PHV or puberty stage, the T1D boys had consistently higher leptin levels than controls. Maximum levels of 4.2ng/ml at 1.5 years before PHV are reached before decreasing to 2.2ng/ml at 3 years after PHV in the T1D boys this contrasts with a plateau (at 2.2ng/ml) in the years before PHV in the control

boys until starting to decline at 1.4 years before PHV to 1.6ng/ml at 1 year after PHV. Leptin levels in both groups of girls increase with time and the T1D girls have higher leptin levels than control girls viewed by years from PHV or by puberty stage. The maximum levels (12.75ng/ml, T1D vs 7ng/ml, controls) were reached by both groups of girls at the end of the observation period of this study but it is likely that the levels continue to increase although the data here is insufficient to allow comment. The pattern of increase from before PHV to after in the two cohorts of girls appears different with a plateau in the control girls around the time of PHV and then a sharp increase, in contrast the T1D girls appear to have a steady increase in leptin levels in relation to PHV. The grouped puberty stage data again shows the marked differences between the two cohorts.

The tendency for leptin levels to increase in girls and fall in boys during puberty reflects the sexual dimorphism in body composition relationships to leptin as girls gain more fat mass and boys more fat free mass during puberty. This has been described by other investigators as well (Blum et al. 1997; Carlsson et al. 1997; Clayton et al. 1997; Garcia-Mayor et al. 1997). Intriguingly, in spite of the continuing decrease in percent body fat in the T1D boys and increasing fat mass in the girls, higher leptin levels are observed in the diabetic subjects than in the controls in both sexes.

Although it is probable that hyperinsulinaemia in the T1D cohort is a major determinant of the elevated leptin levels, sex steroids may well play a role. Testosterone, which is related to fat free mass, appears to have a role in lowering leptin levels although the mechanism is unknown. An inverse relationship of testosterone levels and leptin in boys and men has been observed (Blum et al. 1997; Clayton et al. 1997; Garcia-Mayor et al. 1997;

Mantzoros et al. 1997; Ambrosius et al. 1998) while long-term treatment with testosterone in hypogonadal males decreases leptin concentrations (Jockenhovel et al. 1997). Adan (Adan et al. 1999) treated ten boys with delayed puberty with testosterone for three months and observed a decrease in leptin in spite of weight gain and increasing BMI with no change in insulin concentrations. Palmert et al (Palmert et al. 1998) also observed in boys with central precocious puberty that leptin levels were lower when testosterone was higher before and after GnRH suppression. Wabitsch (Wabitsch et al. 1997) using cultured human adipocytes provided evidence for a direct effect of testosterone on adipose tissue showing that testosterone decreased leptin levels by 62% and suppressed leptin mRNA.

Oestrogen may enhance leptin levels but the findings are not consistent. Shimizu et al (Shimizu et al. 1997b) found that E2 administration increased leptin levels in women and they also observed lower leptin levels in ovariectomised rats compared to normal rats or to other ovariectomised rats given E2 replacement. Casabiell et al (Casabiell et al. 1998) found that E2 increased leptin in omental tissue from women but not men, and several studies reported higher leptin levels during the menstrual cycle in the luteal phase when E2 levels are higher (Shimizu et al. 1997a; Mannucci et al. 1998; Messinis et al. 1998; Riad-Gabriel et al. 1998). Furthermore studies in transsexuals observed that testosterone treatment in females to males decreased leptin but a combination of E2 and antiandrogen increased leptin in male to female, and this was independent of changes in fat mass or the genetic sex (Elbers et al. 1997). Countering these observations, several researchers have not found evidence of an oestrogen stimulating effect on leptin production. Ainslie et al (Ainslie et al. 2001) found no evidence of diminished leptin secretion in ovariectomised rats

confirming the work of Pelleymounter et al (Pelleymounter et al. 1999) whose work in mice led them to conclude that oestradiol does not directly regulate leptin secretion or its effects on fat mass but suggested that the two may interact in an indirect fashion to affect fat utilisation. Havel et al (Havel et al. 1996) reported the same levels of leptin in pre and postmenopausal women if they were matched for fat mass and observed that HRT did not alter leptin levels. In their study of girls with central precocious puberty, Palmert et al (Palmert et al. 1998) observed that oestrogen levels had no discernible effect on leptin levels before, during or after GnRH suppression.

These varied results imply a complex relationship between leptin and oestrogen and suggest that although oestrogen may not have a direct stimulatory role the two may interact in an indirect fashion.

SHBG levels decreased in both sexes and both cohorts of subjects throughout the period studied in agreement with the cross sectional studies of Holly et al (Holly et al. 1989; Holly et al. 1992). T1D children of both sexes had higher levels compared to controls whether by years from PHV or puberty stage. This difference was more marked in the boys compared to the girls. In the boys, the decline appears as an elongated inverted 'S' shape, at 1.75 years before PHV levels are 73nmol/L and decreases until about 1 year after PHV when it levels out. It is similar in the control boys starting at 1.5 years before PHV at 58nmol/L and declining until 1 year after PHV when the rate of decline slows. The pattern of decline in the two cohorts of girls is very different. At 2.5 years before PHV it is near 78nmol/L and at 0.6 years after PHV it is 55nmol/L where it plateaus. Levels in the control girls start at 63nmol/L and appear to stay there until starting to decline at 1.65 years before PHV as far as the data goes after PHV.

The published data are not unanimous on whether levels of SHBG are higher or lower in T1D adolescents compared to control subjects with some observing lower SHBG concentrations (Ebeling et al. 1995; Rudberg and Persson 1995) others reporting equal values in T1D and controls (Meyer et al. 2000) or higher levels in T1D subjects (Djursing et al. 1985; Christensen et al. 1997) and the group of Holly et al (Holly et al. 1992) who observed lower SHBG levels in the T1D children prepubertally but equal concentrations in the pubertal T1D subjects compared to controls.

Sex steroids have long been believed to exert some degree of control over SHBG concentrations (Anderson 1974) and although it was accepted that testosterone decreased SHBG (and this fit with increasing levels of testosterone during puberty and decreasing SHBG levels), it did not entirely support the stimulatory effect that oestrogen was meant to have on SHBG when levels of SHBG decrease during puberty in the face of increasing oestrogen levels. Treated boys with CDGP (Malhotra et al. 1993) and children with adrenarche (Montalto et al. 1989; Balducci et al. 1992; Denburg et al. 2002) were seen to have low SHBG attributed to by increased androgen levels. In a recent article by Sorensen et al negative associations were found between SHBG and testosterone, E2 and DHEAS in boys and DHEAS in girls. This relationship remained after correction for BMI and levels of testosterone and E2. In addition body composition was assessed and negative relationships seen with BMI and percent body fat after adjustment for sex steroids. The conclusion here was that increasing fat mass might be partly responsible for the decrease in levels of SHBG (Sorensen et al. 2007). This is supported in a review by von Schultz and Carlstrom (von Schoultz and Carlstrom 1989) citing earlier work that nutritional status is a strong factor in SHBG regulation with obesity having an inverse

relationship to SHBG levels. In this review they claimed that there was little data to support the claim that endogenous sex steroids were the main physiological regulators but rather that they had a modulating influence with the main regulation being by GH, IGF-I and possibly other growth factors.

There is a body of literature to support the role of insulin in the regulation of SHBG. The report by Plymate in 1988 (Plymate et al. 1988) that insulin decreases SHBG production in the human hepatoma cell line, HepG2, has been subsequently supported by the work of others (Singh et al. 1990; Loukovaara et al. 1995; Kalme et al. 2003) and suggests that we should expect increased SHBG levels as a result of the relative portal hypoinsulinaemia that occurs in T1D subjects. In vivo studies by Yki-Jarvinen et al teased out whether it was the peripheral insulin concentration, estimated portal insulin levels or whole body insulin sensitivity that was the key regulator of SHBG in T1D subjects. They used the euglycaemic hyperinsulinaemic clamp technique to determine whole body insulin sensitivity while portal insulin concentrations were estimated using an established equation. The daily insulin dose/kg (as a marker for long-term insulinisation) was inversely related to SHBG levels and in a multiple regression with insulin sensitivity, portal insulin levels, free testosterone, oestradiol and T4 as independent variables, only the portal insulin concentration was a significant determinant of SHBG concentration (Yki-Jarvinen et al. 1995). In a study suppressing insulin levels in non-diabetic adult male volunteers, Pasquali et al observed increased levels of SHBG and cited this as evidence that insulin inhibits SHBG production (Pasquali et al. 1995).

Free T4 (FT4) was measured in this study and found to be very similar in the control and T1D subjects throughout the study period. There was no significant difference between the two cohorts in either sex when looked at in terms of years from PHV except for the boys at 3 years before PHV when P = 0.05. Since the sample size for the T1D boys was only 7 at this point, however, this may not be a very reliable result. The finding of normal FT4 agrees with the results of Connors et al (Connors et al. 1996) although the findings by an Italian group in 129 T1D children (12.6 ± 3.8 years) compared to 458 healthy age matched controls found that not only were the total T4 levels (92.1 vs 109.2 nmol/L) lower in the T1D children as previously observed by other investigators but the free T4 levels (14.6 vs 17.4pmol/L) were as well (Radetti et al. 1994).

Testosterone levels in the T1D boys are lower than in control boys when considered by years from PHV or puberty stage. In the grouped data, the difference is significant from one year before to 2 years after PHV. Maximum levels achieved were different, T1D reached 16nmol/L four years after PHV where the control boys were at 26nmol/L. By puberty stage, T1D boys had significantly lower concentrations of testosterone compared to controls at all stages except at stage 2.

In the girls, levels of testosterone tended to be lower in the T1D girls compared to control girls from PHV onwards but this difference was only significant one year after PHV, levels between the two cohorts were similar before PHV. Maximum levels of 1.3nmol/L and 1.7nmol/L were achieved in T1D and controls respectively near the end of our observation period. Viewed by puberty stage, levels were lower in T1D girls at all puberty stages from 2-5 but this was only

significant at stage 5. T1D girls had significantly (P = 0.03) higher levels of testosterone at stage 1.

Although the data are not always consistent, most studies report higher levels of testosterone in T1D subjects compared to controls: the early study of Cohen et al, however, found no significant difference in testosterone levels between T1D and control boys within bone age ranges of 11-14.5 years or when matched for puberty stages (Cohen et al. 1984), it should perhaps be noted that there were only 10 T1D boys in this study; the cross sectional study of Meyer et al found no differences between T1D and control adolescents of either sex in levels of total and free testosterone until puberty stage 5 when they observed greater testosterone ($P<0.05$) and free testosterone ($P<0.05$) levels in the T1D girls and boys (Meyer et al. 2000); Salardi et al also observed higher testosterone levels in T1D boys in stage 5 (Salardi et al. 2002); higher testosterone levels in a group of postmenarcheal late pubertal young women with T1D was observed by Rudberg (Rudberg and Persson 1998); and the study by Djursing also found that even those young T1D women with normal cycles had higher testosterone levels than controls which they felt was of ovarian origin (Djursing et al. 1985); this leads of course to the work of both Codner et al and Escobar- Morreale et al whose groups have studied the high prevalence of hyperandrogenicity in T1D women (Escobar-Morreale et al. 2000; Codner et al. 2005). Pasquali et al cite the role of insulin influencing the levels of testosterone in their study of normal men. Suppressing insulin production they observed that testosterone levels decreased and thus claimed that this supports the role that insulin stimulates testosterone production (Pasquali et al. 1995). Perhaps there needs to be a certain level or length of time of chronic hyperinsulinism before the effect on testosterone can be observed. It may be that if our diabetic cohort had been

followed for a longer period that we would have seen higher testosterone levels in our T1D subjects. However to confuse the issue, Tomar et al reported on 50 adult men with T1D aged 23-58 years saying that their total testosterone and free testosterone were in the middle of the normal range and that >90% of their subjects had normal values (Tomar et al. 2006). Although there is no contemporary control group as such, the implication is that these men with T1D do not have elevated testosterone levels.

It may be that the contrasting results are due to a variety of reasons that include small samples in some cases, differing age groups, different disease states, inappropriate controls or different nutritional status/levels of obesity and are perhaps not entirely comparable.

It may be that in our sample of T1D subjects a combination of low IGF-I levels, and hepatic underinsulinisation contribute to low levels of DHEAS and testosterone and higher levels of SHBG.

E2 levels were not measured in the control boys and so no comparison can be made.

In the girls, E2 in the years before PHV was higher in the T1D girls than the control girls and this was significant at two years before. From PHV onwards levels were higher in the control girls with a trend towards significance at PHV ($P = 0.06$) and at 1 year after ($P = 0.07$) and significant at 2 years after, $P = 0.03$. Levels rise more slowly during puberty in T1D girls and reach a maximum 4 years after PHV where the maximum in control girls is achieved at 2.4 years after PHV. By puberty stage, E2 levels were lower in T1D girls from stage 2-5

but none of these differences were significant. Prepubertally however, E2 levels in T1D girls were significantly greater (P = 0.004) than those of control girls.

Data on E2 levels in T1D adolescents are not easy to find. Djursing et al's study on T1D women with and without amenorrhoea commented that normally menstruating diabetic women had normal free and total oestrogens (Djursing et al. 1985). The small study by Zumoff et al followed 5 women with T1D and 8 controls each through a menstrual cycle and observed higher oestradiol levels in the follicular phase and commented that Djursing's study was not comparable since they had not documented normal ovulation (Zumoff et al. 1990). Codner et al studied 56 adolescent girls with T1D and compared to puberty-matched controls found basal levels of oestradiol to be similar at all pubertal stages (Codner et al. 2005).

Chapter 6. Hormonal Associations with Growth and Puberty

6.1 Introduction

An exploration of associations between various biological outcomes including age at onset of puberty, duration of puberty, relative height change and PHV variables with the hormones studied is presented here.

Some differences in puberty and growth have been observed between the two cohorts as well as differences in various hormone levels during puberty. The aim of this chapter is to explore if and to what extent any of these variables play a role in these pubertal events

6.2 Methods

Individual regressions were performed with five different dependent variables: (1) age at pubertal onset (2) PHVSDS (3) HtSDS change (4) duration from stage 2 to PHV and (5) bone age at the start of puberty in T1D children. In the first instance correlation matrices were executed and presented in the Appendix as a source for other researchers but not studied any further here. Complete correlation matrices are in Appendix 8.13. Univariable regressions are presented in the text for all of the considered variables. Those variables that had a $p \leq 0.1$ were entered into a backwards stepwise regression model and the results of these multivariable analyses follow. The procedure was to do this first with sexes kept separate at all times and with the two studies combined together labelled 'all boys' or 'all girls' with diabetes (labelled 'disease') included as a covariate and then secondly to explore the cohorts independently. The R^2 that is reported is the 'adjusted' R^2, the interpretation of which is that it tells us how much of the variance in Y is accounted for if the model had been derived

from the population from which this sample had come (Field 2005). All analyses have been performed on logged hormone values.

6.3 Age of Pubertal Onset and Hormones at Stage 2

6.3.1 Question:

What associations can be demonstrated between the age of the onset of puberty and any of the hormones studied? Do these associations differ between T1D and control children?

6.3.2 Boys

In chapter 3 (section 3.2.2.1) it was observed that there was a significant difference in the age of pubertal onset between the two cohorts (11.3y in controls vs 12.3y in T1D).

6.3.2.1 All Boys

Univariable regressions with Age at Puberty Onset:

Variable	B	se	β	P	N	P adj for disease
1. BMISDS @ G2	.15	.17	.13	.40	46	.53
2. IGF-I	-.31	1.23	-.04	.80	43	.95
3. A4	-.45	.70	-.10	.53	40	.40
4. DHEAS	.46	.52	.14	.38	40	.27
5. E2	Not available for controls					
6. leptin	1.96	.87	.40	.03	29	.73
7. SHBG	.73	1.31	.09	.58	40	1.00
8. testosterone	.16	.44	.05	.73	43	.63
9. FAI	-.02	.41	-.01	.95	40	.83

In a multivariable regression entering both leptin and 'disease' with age at puberty onset as the dependent variable (N=29), only *'disease' has a significant effect: B = 1.12 ± 0.31, P = 0.001 with β = 0.57; P for the model = 0.001 and R^2 = 30.3%*. Thus the presence of T1D in boys results in a delay in the onset of puberty.

6.3.2.2 Control Boys

Univariable Regressions with Age at Puberty Onset:

Variable	B	se	β	P	N
1. BMISDS @G2	.12	.20	.13	.55	23
2. IGF-I	-.60	1.97	-.07	.76	22
3. A4	-.57	.70	-.18	.43	21
4. DHEAS	1.22	.52	.48	.03	21
5. E2	Not available				
6. leptin	1.12	.90	.30	.23	18
7. SHBG	-1.30	1.44	-.20	.38	21
8. testosterone	.73	.66	.24	.28	22
9. FAI	.65	.58	.25	.27	21

DHEAS has a significant effect in control boys on the age of pubertal onset, B = 1.22 ± 0.52, P = 0.03 with β = 0.48, P for model = 0.03 and R^2 = 18.4%.

6.3.2.3 T1D Boys

Univariable regressions with Age at Puberty Onset:

Variable	B	se	β	P	N
1. BMISDS @ G2	-.50	.27	-.38	.08	23
2. IGF-I	.41	1.38	.07	.77	21
3. A4	-.45	1.48	-.07	.76	19
4. DHEAS	-.75	.80	-.22	.36	19
5. E2	.10	.48	.05	.84	19
6. leptin	-1.21	2.35	-.17	.62	11
7. SHBG	2.79	2.10	.31	.20	19
8. testosterone	-.11	.51	-.05	.83	21
9. FAI	-.28	.48	-.14	.57	19
10. HbA1c @ G2	.07	.14	.10	.64	23
11. ins/kg @ G2	-.33	1.30	-.06	.80	23
12. bone age @G2	.48	.13	.71	.003	18

In a multivariable regression entering both BMISDS and bone age@G2 with age at puberty onset as the dependent variable (N=18), only *bone age at G2 is a significant determinant: B = 0.48 ± 0.13, P = 0.003 with β = 0.71 and R^2 = 46.6%.*

As might be expected, there is a strong and highly significant association of bone age and chronological age at the start of puberty. Removing bone age

and looking at only BMISDS (N=23): *B = - 0.50 ± 0.27, P = 0.08 with β = - 0.38 and R^2 = 10.1%.*

6.3.2.4 Summary

In 'all boys', of the various variables considered, only leptin has a significant and positive correlation to age at pubertal onset. In a model adjusting for disease presence, 'disease' is significant indicating that having T1D in the boys is associated with a delayed pubertal onset of 1.12 years, P = 0.001 with this accounting for 30.3% of the variance in age of onset.

In the control boys, DHEAS has a significant association with the age at the start of puberty. Lower levels of DHEAS at G2 are associated with a younger age of pubertal onset. In the T1D boys there is no correlation between any of the hormones studied and the age at the start of puberty but bone age at this time is robustly correlated to the chronological age, r = 0.71, P = 0.003.

BMISDS has a negative correlation that missed reaching significance, P = 0.08, and in a multivariable model with bone age it does not exert a significant effect while bone age alone accounts for 46.6% of the variance.

6.3.3 Girls

6.3.3.1 All Girls

Univariable regressions with Age at Puberty Onset:

Variable	B	se	β	P	N	P adj for disease
1. BMISDS@B2	-.40	.12	-.39	.002	64	.004
2. IGF-I	1.33	.86	.23	.13	44	.14
3. A4	.78	.65	.20	.24	36	.30
4. DHEAS	-.16	.33	-.08	.63	36	.47
5. E2	.99	.34	.41	.006	43	.006
6. leptin	-.12	.68	-.04	.86	21	.62
7. SHBG	-.02	.97	-.003	.99	36	.95
8. testosterone	.74	.53	.22	.17	43	.16
9. FAI	.32	.47	.11	.51	36	.55

In a multivariable regression entering BMISDS, E2 and 'disease' with age at puberty onset as the dependent variable (N=42), *both BMISDS (B = - 0.37 ± 0.10, P <0.0005 with β - 0.47) and E2 (B = 0.86 ± 0.30, P = 0.006 with β = 0.36) are independent significant determinants* while 'disease' did not have a significant effect. P of model <0.0005 and R^2 = 36.2%.

6.3.3.2 Control Girls

Univariable regressions with Age at Puberty Onset:

Variable	B	se	β	P	N
1. BMISDS	-.47	.15	-.44	.003	43
2. IGF-I	1.02	1.42	.16	.48	23
3. A4	1.77	1.41	.31	.23	17
4. DHEAS	-.72	.69	-.26	.31	17
5. E2	1.09	.43	.49	.02	23
6. leptin	-1.56	1.84	-.25	.41	13
7. SHBG	-.23	2.29	-.03	.92	17
8. testosterone	1.13	.73	.32	.14	23
9. FAI	.58	.72	.20	.80	17

In a multivariable regression entering BMISDS and E2 with age at puberty onset as the dependent variable (N=23), *both BMISDS and E2 are independent*

significant determinants: B = - 0.56 ± 0.12, P <0.0005 with β - 0.65 and B = 0.83 ± 0.30, P = 0.01 with β = 0.37 respectively; P of model <0.0005 and R^2 = 61.1%.

6.3.3.3 T1D Girls

Univariable regressions with Age at Puberty Onset:

Variable	B	se	β	P	N
1. BMISDS@B2	.002	.23	.002	.99	21
2. IGF-I	1.58	1.02	.33	.14	21
3. A4	.13	.66	.05	.85	19
4. DHEAS	.06	.34	.05	.86	19
5. E2	.15	.86	.04	.87	20
6. leptin	.45	.82	.22	.61	8
7. SHBG	.19	.86	.05	.83	19
8. testosterone	-.24	.74	-.08	.75	20
9. FAI	-.34	.62	-.13	.59	19
10. HbA1c	.24	.09	.53	.01	21
11. ins/kg	1.18	.50	.47	.03	21
12. BA@ B2	.20	.13	.36	.16	17

In a multivariable regression entering both HbA1c and ins/kg @ B2 with age at puberty onset as the dependent variable (N=21), *only HbA1c at B2 is a significant determinant, B = 0.24 ± 0.09, P = 0.01 with β = 0.53; P of model = 0.01, R^2 = 24.6%.*

6.3.3.4 Summary

In 'all girls' both BMISDS and E2 at the start of puberty have significant correlations with the age at pubertal onset. In a multivariable model they are both independently significant determinants and account for 36.2% of the variance and no 'disease' effect is observed on age of pubertal onset as it was in the boys.

In the control girls, again both BMISDS and E2 at the start of puberty are associated with the age of pubertal onset. In the multivariable regression, BMISDS has a major effect with 61.1% of the variance explained by the two

variables. Thus girls without T1D who have a greater BMISDS at the start of puberty will tend to have an earlier puberty adjusting for their E2 levels at the start of puberty.

In the T1D girls, as in the T1D boys, none of the hormones at the start of puberty were identified as playing a significant role in the age of pubertal onset. In these girls, however, both HbA1c and insulin dose at the start of puberty were related to the age of puberty onset. In a model adjusting for each other only HbA1c remains a significant factor, accounting for 24.6% of the variance. Thus girls with poorer metabolic control will tend to have a later pubertal onset, for every 1% increase in HbA1c, age at puberty will be delayed by 0.24 of a year (3 months). Surprisingly BMISDS displays no association with the age of pubertal onset in the T1D girls.

6.4 Duration Stage 2-PHV

6.4.1 Question

Is it possible to identify if BMI or any of the hormones at the start of puberty has an influence on this duration and how this may differ between the two cohorts.

6.4.2 Boys

Control boys were previously (chapter 3, section 3.2.2.4) observed to have a significantly longer duration from the onset of puberty to PHV than boys with T1D, 2.55 vs 1.84 years.

6.4.2.1 All Boys

Univariable regressions with duration from puberty onset to PHV:

Variable	B	se	β	P	N	P adj for disease
1. BMISDS @ G2	-.33	.14	-.33	.02	46	.13
2. IGF-I	-1.77	1.11	-.24	.12	43	.05
3. A4	-.19	.64	-.05	.77	40	.84
4. DHEAS	-.68	.47	-.23	.16	40	.11
5. E2	Not available for control boys					
6. leptin	-1.59	.68	-.41	.03	29	.06
7. SHBG	.90	1.19	.12	.46	40	.22
8. testosterone	-1.27	.36	-.48	.001	43	<0.0005
9. FAI	-1.02	.33	-.45	.004	40	.001

Since FAI and testosterone are highly correlated, r = 0.94, P<0.0005; FAI has been omitted from the ensuing multivariate analysis.

In a multivariable regression entering testosterone, BMISDS, leptin and 'disease' with duration from G2 to PHV as the dependent variable (N=29), *both testosterone (B = - 1.38 ± 0.50, P = 0.01 with β = - 0.51) and 'disease' (B = - 0.69 ± 0.29, P = 0.03 with β = - 0.45) are significant independent determinants with P of the model = 0.02 and R^2 = 20.5%.*

6.4.2.2 Control Boys

Univariable regressions with duration from puberty onset to PHV:

Variable	B	se	β	P	N
1. BMISDS @ G2	-.31	.20	-.33	.13	23
2. IGF-I	-3.94	1.73	-.45	.03	22
3. A4	.20	.70	.06	.78	21
4. DHEAS	-.83	.55	-.33	.14	21
5. E2	Not available				
6. leptin	-2.97	.82	-.67	.002	18
7. SHBG	1.20	1.41	.19	.41	21
8. testosterone	-1.49	.57	-.50	.02	22
9. FAI	-1.13	.53	-.44	.045	21

FAI and testosterone are correlated, r = 0.89, P < 0.0005 as well as FAI and leptin, r = 0.87, P<0.0005 and thus FAI has been omitted from the multivariate analysis.

Entering testosterone, leptin and IGF-I into a multivariable regression with duration from G2 to PHV as the dependent variable (N=18), *only testosterone has a significant effect, B = - 1.67 ± 0.45, P = 0.002 with β = - .68; P of the model = 0.002 and R^2 = 43.3%.*

6.4.2.3 T1D Boys

Univariable regressions with duration from puberty onset to PHV:

Variable	B	se	β	P	N
1. BMISDS @ G2	-.12	.26	-.10	.65	23
2. IGF-I	-1.16	1.30	-.20	.39	21
3. A4	-1.43	1.37	-.25	.31	19
4. DHEAS	-.52	.77	-.16	.51	19
5. E2	.36	.43	.20	.41	19
6. leptin	.85	1.81	.15	.65	11
7. SHBG	1.92	2.05	.22	.36	19
8. testosterone	-1.18	.41	-.56	.009	21
9. FAI	-1.08	.38	-.57	.01	19
10. HbA1c @ G2	-.25	.11	-.44	.04	23
11. ins/kg @ G2	-.41	1.15	-.08	.73	23
12. bone age @ G2	.04	.16	.06	.80	18

Testosterone and FAI are highly correlated, r = 0.98, P <0.0005 and so FAI has been left out of the multivariate analysis. Entering testosterone and HbA1c into a multivariable regression with duration G2 to PHV as the dependent variable (N=21), *testosterone was a significant determinant, B = - 0.92 ± 0.41, P = 0.04 with β = -.43 with a trend towards a significant effect from HbA1c, B = - 0.20 ± 0.11, P = 0.09 with β = -.35; P of the model = 0.008 and R^2 = 34.8%.*

6.4.2.4 Summary

In 'all boys', BMISDS, FAI, leptin, and testosterone all individually have negative correlations with the duration from the start of puberty to PHV. In a multivariable

model, testosterone and 'disease' are independently significant determinants of this duration. Not having diabetes and adjusting for levels of testosterone at the start of puberty, is associated with a longer duration of puberty from G2 to PHV. Higher levels of testosterone at the start of puberty, controlling for the presence/absence of 'disease', are related to a shorter duration.

In control boys, FAI, leptin, testosterone and IGF-I (but not BMISDS) are all negatively correlated to the G2-PHV duration. Since there are a number of highly related and significant correlations, IGF-I, leptin and testosterone were entered into the multivariable model and testosterone levels at the start of puberty remained significantly negative and so higher levels of testosterone at G2 are associated with a shorter duration from G2 to PHV.

In T1D boys, testosterone and HbA1c were both negative and significant independent determinants of the duration from G2 to PHV thus higher testosterone levels at the start of puberty, adjusting for HbA1c levels, are related to a shorter duration from the start of puberty to PHV. There was a trend for HbA1c to have an effect and so adjusting for levels of testosterone, a higher HbA1c at the start of puberty will tend to be associated with a shorter G2-PHV duration.

6.4.3 Girls

It was previously observed (chapter 3, section 3.2.3.4) that there was no difference in the duration from B2 to PHV between the T1D girls and control girls.

6.4.3.1 All Girls

Univariable regressions with duration from puberty onset to PHV:

Variable	B	se	β	P	N	P adj for disease
1. BMISDS @ B2	.27	.11	.32	.02	53	.01
2. IGF-I	-2.60	.75	-.47	.001	44	.001
3. A4	-.34	.70	-.08	.63	36	.44
4. DHEAS	.36	.35	.17	.32	36	.42
5. E2	-.88	.37	-.35	.02	43	.03
6. leptin	-.19	.75	-.06	.80	21	.40
7. SHBG	.90	1.19	.12	.46	36	.86
8. testosterone	-.92	.55	-.26	.10	43	.12
9. FAI	-.37	.50	-.13	.46	36	.43

Entering BMISDS, E2, IGF-I, testosterone at B2 and 'disease' into a multivariable regression with duration B2 to PHV as the dependent variable (N=43), *both BMISDS (B = 0.24 ± 0.11, P = 0.03 with β =0.29) and IGF-I (B = -2.52 ± 0.76, P = 0.002 with β = -.44) are independent significant determinants with P of the model = 0.001 and R^2 = 25.3%.*

6.4.3.2 Control Girls

Univariable regressions with duration from puberty onset to PHV:

Variable	B	se	β	P	N
1. BMISDS @ B2	.36	.14	.44	.01	32
2. IGF-I	-2.79	1.22	-.45	.03	23
3. A4	-.92	1.36	-.17	.51	17
4. DHEAS	.61	.65	.24	.36	17
5. E2	-.91	.42	-.43	.04	23
6. leptin	1.93	1.62	.34	.26	13
7. SHBG	.32	2.13	.04	.88	17
8. testosterone	-1.35	.67	-.40	.06	23
9. FAI	-.88	.65	-.33	.19	17

Entering BMISDS, E2, IGF-I and testosterone at B2 into a multivariable regression with duration B2 to PHV as the dependent variable (N=23), *both BMISDS (B = 0.37 ± 0.14, P = 0.02 with β = 0.45) and IGF-I (B = - 2.63 ± 1.08,*

P = 0.02 with β = -.42) are independent significant determinants with P of the model = 0.006 and R^2 = 34.1%.

6.4.3.3 T1D Girls

Univariable regressions with duration from puberty onset to PHV:

Variable	B	se	β	P	N
1. BMISDS @ B2	.15	.23	.15	.52	21
2. IGF-I	-2.50	.91	-.53	.01	21
3. A4	-.39	.90	-.11	.67	19
4. DHEAS	.09	.47	.05	.84	19
5. E2	-.49	1.13	-.10	.67	20
6. leptin	.10	1.71	.02	.96	8
7. SHBG	-.42	1.18	-.09	.73	19
8. testosterone	.41	.99	.10	.69	20
9. FAI	.64	.84	.18	.46	19
10. HbA1c @ B2	-.15	.10	-.34	.13	21
11. ins/kg @ B2	-1.19	.50	-.48	.03	21
12. bone age @ B2	-.09	.20	-.12	.66	17

Entering IGF-I and insulin dose at B2 into a multivariable regression with duration B2 to PHV as the dependent variable (N=21), *IGF-I (B = - 2.46 ± 0.91, P = 0.01 with β = -.53) is a significant determinant with P of the model = 0.01 and R^2 = 23.9%.*

6.4.3.4 Summary

In 'all girls' and control girls, IGF-I levels, oestradiol and BMISDS at the start of puberty were each related to the B2-PHV duration. In a multivariable model, both IGF-I and BMISDS remained as independent significant determinants of the duration from B2 to PHV. There is no effect of 'disease'. IGF-I is negative, thus higher levels of IGF-I at the start of puberty, adjusting for BMISDS, are associated with a shorter duration from the onset of puberty to PHV. BMISDS on the other hand has a positive relation with this duration and so those girls with a higher BMISDS at the start of puberty, adjusting for IGF-I levels, will take longer to go from B2 to PHV.

In the T1D girls insulin dose and IGF-I at the start of puberty are related to this B2-PHV duration but only IGF-I remained as a significant factor in the multivariable model. Thus higher levels of IGF-I at the start of puberty are associated with a shorter duration from B2 to PHV and this explains 23.9% of the variance on its own.

6.5 HtSDS Change from Puberty Stage 2 to PHV

6.5.1 Calculation note

Ht SDS has been calculated by using an averaged height for each child at the time of stage 2 and relating that height to the sex appropriate mean and sd for height from the revised growth charts of Tanner Buckler (Castlemead Publications) (Tanner and Buckler 1997) which compared well to the means of the Freeman et al 1995 British references (Freeman et al. 1995) at the ages reported by Marshall and Tanner for stage 2 (Marshall and Tanner 1969; Marshall and Tanner 1970). An averaged height was used since most children were seen several times in stage 2 and the Marshall and Tanner references actually state they used a point midway between each stage since it was impossible to ever know exactly when a child entered a particular puberty stage. The height SDS was calculated as: (subject's height-mean height at age of ref stage 2)/SD at age ref stage 2. HtSDS at PHV has been calculated for each child by looking at their height at their age of PHV and using the mean and SD at that age from the Tanner Buckler charts which again for the boys were very similar to the Freeman values at the same age but for the girls were not and so the Tanner height values were chosen since they were felt to better reflect values based on a longitudinal trajectory. The hormones for these analyses are all logged and averaged from stage 2-PHV. In the T1D subjects, insulin dose

and HbA1c have been explored both at stage 2 and averaged (av) from stage 2 to PHV.

6.5.2 Question:

Which of the variables chosen affect the change in HtSDS (which could increase or decrease) from the start of puberty to peak height velocity in the four groups? Are there differences in the associations between T1D and control children?

6.5.3 Boys

6.5.3.1. All Boys

Univariable regressions with HtSDS change:

Variable	B	se	β	P	N	P adj for disease
1. BMISDS @ G2	-.29	.10	-.39	.008	45	.05
2. IGF-I	-.09	1.09	-.01	.94	44	.52
3. A4	.25	.55	.07	.66	43	.79
4. DHEAS	-.33	.33	-.16	.31	43	.09
5. E2	Not available for controls					
6. leptin	-1.43	.54	-.43	.01	34	.06
7. SHBG	1.12	.82	.21	.18	43	.02
8. testosterone	-.51	.34	-.23	.14	44	.05
9. FAI	-.44	.27	-.25	.11	43	.03
10. age@G2	-.26	.09	-.41	.006	45	.05
11. duration G2-PHV	.56	.07	.78	<0.0005	45	<0.0005

In a multivariable model entering BMISDS @ G2, age @ G2, duration from G2-PHV, leptin and 'disease' with HtSDS change as the dependent variable, (N=34), results in *both age at G2 and duration from G2 to PHV being independently related to HtSDS change in 'all boys': B = - 0.17 ± 0.07, P = 0.03 with β = - 0.25 and B = 0.60 ± 0.09, P<0.0005 with β = 0.71 respectively; P of the model <0.0005 and R^2 = 65.2%.*

The covariate 'duration from G2 to PHV' overwhelms the model, if it is left out and a second model is set up with BMISDS @ G2, age @ G2, leptin and

'disease' entered with HtSDS change as the dependent variable (N=34), *the significant determinant is age at G2 (B = - 0.29 ± 0.10, P = 0.009 and β = - 0.43) with a trend towards a significant effect of BMISDS at G2 (B = - 0.21 ± 0.11, P = 0.07 and β = - 0.28), P of the model = 0.005 and R^2 = 24.6%.*

There is no 'disease' effect.

6.5.3.2 Control Boys

Univariable Regressions with HtSDS change:

Variable	B	se	β	P	N
1. BMISDS @G2	-.24	.15	-.34	.11	23
2. IGF-I	-.70	1.74	-.09	.40	22
3. A4	.17	.65	.06	.80	21
4. DHEAS	-.82	.46	-.38	.09	21
5. E2	Not available				
6. leptin	-2.31	.76	-.60	.007	19
7. SHBG	1.45	1.17	.27	.23	21
8. testosterone	-.52	.61	-.19	.40	22
9. FAI	-.71	.58	-.27	.23	21
10. age@G2	-.40	.14	-.53	.01	23
11. duration G2-PHV	.56	.11	.74	<0.0005	23

In a multivariable regression entering DHEAS, leptin, age @ G2 and duration G2-PHV with HtSDS change as the dependent variable (N=18), *the significant determinant is duration G2-PHV: B = 0.61 ± 0.14, P = 0.001 and β = 0.68 with age at G2 not quite significant B = - 0.31 ± 0.17, P = 0.09 and β = - 0.29; with the P of the model <0.0005 and R^2 = 59.9%.*

Again, leaving out the covariate, 'duration G2-PHV', the outcome from entering DHEAS, leptin and age@G2 with HtSDS change as the dependent variable, (N=18) results in leptin as the only significant determinant: *B = - 2.34 ± 0.78, P = 0.008 and β = - 0.60; with P of the model = 0.008 and R^2 = 32.2%.*

6.5.3.3 T1D Boys

Univariable regressions with HtSDS change:

Variable	B	se	β	P	N
1. BMISDS @G2	-.21	.19	-.24	.29	22
2. IGF-I	-.68	1.34	-.11	.62	22
3. A4	-.04	1.06	.008	.97	22
4. DHEAS	-.24	.44	-.12	.60	22
5. E2	.34	.29	.25	.26	22
6. leptin	.43	1.23	.10	.73	15
7. SHBG	2.48	1.07	.46	.03	22
8. testosterone	-.71	.36	-.41	.06	22
9. FAI	-.57	.27	-.43	.05	22
10. age @ G2	-.04	.14	-.06	.79	22
11. duration G2-PHV	.53	.10	.76	<0.0005	22
12. HbA1c av G2-PHV	-.15	.08	-.39	.07	22
13. HbA1c @G2	-.25	.07	-.62	.002	22
15. ins/kg av G2-PHV	-1.25	.85	-.31	.16	22
16. ins/kg @ G2	-1.05	.78	-.29	.19	22
17. bone age@G2	-.06	.12	-.13	.61	17

NB: SHBG is a carrier protein and as such it may be debated whether it really has anything directly to do with growth. Taking this approach, SHBG has been presented in the univariable tables for interest but not considered in multivariable analyses. FAI as a derived ratio (testosterone/SHBG x 100) has also not been considered in any multivariable analyses.

In a multivariable regression entering testosterone, duration G2-PHV and HbA1c @ G2 with HtSDS change as the dependent variable (N=22), *both HbA1c at G2 (B = - 0.14 ± 0.06, P = 0.02 and β = - 0.36) and duration G2-PHV (B = 0.42 ± 0.10, P <0.0005 and β = 0.61) are significant with P of the model <0.0005 and R^2 = 65.1%:*

The covariate, 'duration G2-PHV', again overwhelms the model, if it is omitted, the outcome from entering testosterone and HbA1c @ G2 in a multivariable model with HtSDS change as the dependent variable (N=22) leaves just *HbA1c at G2 as significant: B = - 0.25 ± 0.07, P = 0.002 and β = - 0.62) with P of the model = 0.002 and R^2 = 35.6%.*

6.5.3.4 Summary

In 'all boys' in univariable correlations, BMISDS at G2, age at G2 and leptin (averaged from G2 to PHV) are all negatively related to HtSDS change (P = 0.008, P = 0.006 and P = 0.01 respectively) while duration from G2 to PHV is highly positively associated with HtSDS change, P<0.0005. Testosterone and FAI are both negative and significantly related to HtSDS change when adjusted for 'disease' (P = 0.05 and P = 0.03 respectively). In a multivariable model including age and BMISDS at the start of puberty, duration, leptin and 'disease'; age and duration were independently related with the HtSDS change that occurs between G2 and PHV. A longer duration is associated with a greater relative height gain when adjusted for the age of pubertal onset, whereas a younger age at G2 is associated with a greater relative height gain. This model explains 65.2% of the variance in the outcome. The 'duration' covariate overwhelms the model and if it is left out BMISDS and age at the start of puberty are both negative and independently associated with relative height gain and this model explains 24.6% of the variance.

In control boys, in univariable correlations, leptin levels, DHEAS and age at G2 are all negatively related to HtSDS change (P = 0.007, P = 0.09 and P = 0.01 respectively) while duration G2-PHV is positively associated with HtSDS change (p<0.0005). In multivariable regression, including age at G2, duration, DHEAS and leptin, both age at G2 and duration are independently associated with HtSDS change, R^2 = 59.9%. The younger the age of G2, adjusting for the interval G2-PHV, the greater the relative height gain, and the longer the duration from the start of puberty to PHV, adjusting for the age at the start of puberty, the greater is the relative height gain. Interestingly, if again duration is omitted from this model, leptin remains as the only significant determinant, explaining 32.2%

of the variance and lower leptin levels over this time are associated with greater relative height gain.

In the T1D boys in a univariable correlation, HbA1c at the start of puberty and testosterone and FAI averaged from pubertal onset to PHV are all negatively related to HtSDS change (P = 0.002, P = 0.06 and P = 0.05 respectively) while age of pubertal onset is not associated with HtSDS change. Duration from the start of puberty to PHV is again robustly associated with the HtSDS change during this time. If HbA1c, testosterone and duration are entered into a multivariable model, HbA1c and duration are both significant determinants. This model explains 65.1% of the variance. Thus in T1D boys, the higher HbA1c at the start of puberty (ie poorer control), adjusting for pubertal duration, is associated with less relative height gain. If the overwhelming influence of duration is removed then only HbA1c is significant and this explains 35.6% of the variance in HtSDS change.

6.5.4 Girls

6.5.4.1 All Girls

Univariable Regressions with HtSDS change:

Variable	B	se	β	P	N	P adj for disease
1. BMISDS @ B2	.20	.11	.25	.08	52	.02
2. IGF-I	-.50	.99	-.08	.61	42	.44
3. A4	.77	.64	.21	.24	35	.56
4. DHEAS	.59	.31	.32	.06	35	.14
5. E2	-.17	.42	-.06	.69	42	.43
6. leptin	.25	.53	.09	.64	27	.12
7. SHBG	-1.16	.98	-.20	.24	35	.32
8. testosterone	-.09	.53	-.03	.86	42	.84
9. FAI	.49	.49	.17	.32	35	.42
10. age@B2	-.56	.09	-.66	<0.0005	52	<0.0005
11. duration B2-PHV	.85	.08	.82	<0.0005	51	<0.0005

In a multivariable model, entering BMISDS@B2, age@B2, duration B2-PHV, DHEAS and 'disease' with HtSDS as the dependent variable, (N=35) *both duration B2-PHV and age at puberty onset were highly significant* with both BMISDS at B2 and 'disease' showing a trend towards significance:

Variable	B	se	β	t	P	P of Model	R^2 adj
duration B2-PHV	.61	.09	.61	7.02	<0.0005	<0.0005	82.2%
'disease'	-.23	.12	-.16	1.94	.06		
age @ B2	-.39	.08	-.46	-4.85	<0.0005		
BMISDS @ B2	-.11	.06	-.16	-1.77	.09		

Leaving out the covariate, 'duration B2-PHV', which has an overwhelming influence, and in 'all girls' is related to age at the start of puberty ($r = - 0.58$, $p<0.0005$) although admittedly not enough to show up in the collinearity statistics/diagnostics of SPSS.

The results from this model entering BMISDS@B2, age@B2, DHEAS and 'disease' with HtSDS as the dependent variable (N=35), gives *both age at puberty onset, $B = - 0.61 \pm 0.10$, $P <0.0005$ with $\beta = - 0.73$ and 'disease', $B = - 0.50 \pm 0.17$, $P = 0.007$ with $\beta = - 0.33$. as significant independent determinants of HtSDS change from puberty onset to PHV with P of the model <0.0005 and $R^2 = 54.7\%$.*

6.5.4.2 Control Girls

Univariable regressions with HtSDS change:

Variable	B	se	β	P	N
1. BMISDS @ B2	.36	.13	.46	.009	31
2. IGF-I	1.66	1.98	.18	.41	22
3. A4	-.05	1.35	-.01	.97	16
4. DHEAS	.90	.57	.39	.14	16
5. E2	-.79	.45	-.37	.09	22
6. leptin	1.64	.85	.46	.07	16
7. SHBG	-3.00	1.69	-.43	.10	16
8. testosterone	-.71	.64	-.24	.28	22
9. FAI	-.10	.69	-.04	.89	16
10. age @ B2	-.57	.10	-.73	<0.0005	31
11. duration B2-PHV	.84	.12	.81	<0.0005	30

In a multivariable regression entering BMISDS@ B2, E2, leptin, age@B2 and duration B2-PHV with HtSDS change as the dependent variable (N=16) results in *both age at puberty onset (B = - 0.37 ± 0.17, P = 0.045 and β = - 0.47) and duration B2-PHV (B = 0.40 ± 0.18, P = 0.04 and β = 0.47) as significant with P of the model <0.0005 and R^2 = 75.5%.*

Although BMISDS@B2 and leptin are related, r = 0.53, P = 0.04, this is not strong enough to conflict in the model and so both have been left in. However, the duration B2-PHV is correlated with age at B2 (r = - 0.63, p<0.0005), and although not enough to affect the collinearity statistics of the model, duration was omitted in a second multivariable model and entering BMISDS @ B2, E2, leptin, and age @ B2 with HtSDS change as the dependent variable (N=16) results in: *age at puberty onset is significant: B = - 0.67 ± 0.12, P <0.0005 and β = - 0.84, P of the model <0.0005 and R^2 = 68.5%.*

6.5.4.3 T1D Girls

Univariable regressions with HtSDS change:

Variable	B	se	β	P	N
1. BMISDS @ B2	.04	.22	.04	.86	21
2. IGF-I	-1.60	1.03	-.34	.14	20
3. A4	.73	.90	.19	.43	19
4. DHEAS	.28	.41	.16	.51	19
5. E2	2.49	.93	.53	.02	20
6. leptin	.52	1.33	.13	.70	11
7. SHBG	-.01	1.17	-.003	.99	19
8. testosterone	1.10	.82	.30	.19	20
9. FAI	1.12	.69	.37	.12	19
10. age @ B2	-.72	.15	-.75	<0.0005	21
11. duration B2-PHV	.83	.12	.85	<0.0005	21
12. HbA1c av B2-PHV	-.03	.12	-.05	.83	21
13. HbA1c @ B2	-.20	.09	-.45	.04	21
15. ins/kg av B2-PHV	-1.01	.50	-.43	.06	21
16. ins/kg @ B2	-1.33	.46	-.55	.01	21
17. bone age@ B2	-.20	.16	-.31	.23	17

A multivariable regression entering E2, HbA1c @ B2, ins/kg @ B2, age@B2 and duration B2-PHV with HtSDS change as the dependent variable (N=20) *results in both age at puberty onset (B = - 0.40 ± 0.12, P = 0.003 and β = - 0.46) and duration B2-PHV (B = 0.53 ± 0.13, P = 0.001 and β = 0.55) as significant determinants with P of the model <0.0005 and* R^2 *= 78.7%.*

In the T1D girls age at pubertal onset and duration from B2-PHV are correlated, r = -0.58, P = 0.006, and again while not enough to appear in the collinearity statistics, the effect of duration is strong and so it has been left out to further explore other influences. The results from entering E2, HbA1c @ B2, ins/kg @ B2 and age @ B2 with HtSDS change as the dependent variable (N=20):

Variable	B	se	β	t	P	P of Model	R^2 adj
age @ B2	-.44	.15	-.50	-2.96	.009	<0.0005	68.4%
E2	1.40	.65	.30	2.15	.05		
HbA1c @ B2	-.12	.07	-.29	-1.85	.08		

6.5.4.4 Summary

In 'all girls' in univariable correlations, age at B2 and duration from B2 to PHV are both highly significantly associated (P<0.0005) with HtSDS change between the start of puberty and PHV. Both DHEAS averaged over the time period and BMISDS at B2 show a slight relationship (P = 0.06 and P = 0.08 respectively) although BMISDS became significant when adjusted for 'disease' (P = 0.02). In a multivariable model, entering age and BMISDS at B2, DHEAS, duration B2-PHV and 'disease', both age at puberty onset and duration from B2 to PHV are highly significant, P<0.0005 while both 'disease' and BMISDS, adjusted for the other covariates, show a trend towards a relation with HtSDS change. This model is highly significant (P <0.0005) and explains 82.2% of the variance. However, as in the boys, the 'duration' covariate overwhelms the model and if it is omitted then both age at the onset of puberty and 'disease' remain the significant covariates in a model that now explains 54.7% of the variance. Thus not having diabetes is associated with a greater relative height gain adjusting for the age of B2. And an earlier age at B2, allowing for disease status, is associated with a greater relative height gain.

In control girls in univariable correlations, age at B2 and duration from B2 to PHV are both highly significantly associated with Ht SDS change as is BMISDS at B2 while leptin and E2 also show some association. In a multivariable model entering these five covariates, age at B2 and duration B2-PHV are independently associated with HtSDS change. This model is highly significant and explains 75.5% of the variance. Thus earlier puberty and longer duration are both independently associated with a greater relative height gain adjusting for each other.

In T1D girls in univariable correlations with HtSDS change, the following are significant: age at B2, duration B2-PHV, HbA1c at B2, ins/kg at B2 and averaged levels of E2. When these covariates are entered into a multivariable model: age at B2 and duration are again significant explaining 78.7% of the variance. If duration is omitted, then age at B2, E2 levels, and HbA1c remain in the model that explains 68.4% of the variance. Each one is associated with a greater relative height gain adjusting for the other two. Thus earlier puberty onset in T1D girls (adjusting for averaged levels of E2 as well as HbA1c values at B2), higher average levels of E2 (allowing for age at B2 and HbA1c values) and lower HbA1c (ie better control, adjusting for age at B2 and levels of E2) are all associated with greater relative height gain from B2 to PHV.

6.6 PHVSDS and hormones/biological variables at PHV

6.6.1 Question:

What hormones or other biological variables i.e., BMISDS (bone age, insulin dose and HbA1c in the T1D cohort) at PHV are associated with PHVSDS? Do these associations differ between T1D and control children?

6.6.2 Boys

6.6.2.1 All Boys

Univariable regressions with PHVSDS:

Variable	B	se	β	P	N	P adj for disease
1. BMISDS @PHV	-.21	.19	-.16	.28	46	.38
2. IGF-I	2.98	1.49	.29	.05	45	.06
3. A4	2.41	.79	.43	.004	44	.008
4. DHEAS	-.18	.52	-.05	.74	44	.42
5. E2	Not available in control boys					
6. leptin	-1.00	1.02	-.17	.33	34	.49
7. SHBG	1.09	1.05	.16	.30	44	.10
8. testosterone	1.15	.55	.30	.04	46	.06
9. FAI	.46	.47	.15	.34	44	.64

In a multivariable regression entering testosterone, A4, IGF-I and 'disease' with PHVSDS as the dependent variable (N=43), *both A4 (B = 2.20 ± 0.70, P = 0.006 with β = 0.40) and IGF-I (B = 2.50 ± 1.39, P = 0.08 with β = 0.25)* are independent determinants although IGF-I does not quite reach significance; *P of the model = 0.004, R^2 = 20.5%*

6.6.2.2 Control Boys

Univariable regressions with PHVSDS:

Variable	B	se	β	P	N
1. BMISDS	-.18	.27	-.15	.51	23
2. IGF-I	2.28	3.42	.14	.51	23
3. A4	2.11	1.08	.40	.06	22
4. DHEAS	-.70	.88	-.18	.44	22
5. E2	Not available				
6. leptin	-1.06	1.37	-.18	.45	20
7. SHBG	3.10	1.58	.40	.06	22
8. testosterone	3.02	1.06	.53	.009	23
9. FAI	.67	1.19	.12	.58	22

In a multivariable regression entering testosterone and A4, with PHVSDS as the dependent variable (N=22), *testosterone is a significant determinant: B = 3.77 ± 1.05, P = 0.002 with β = 0.63; P of the model = 0.002 and R^2 = 36.2%.*

6.6.2.3 T1D Boys

Univariable regressions with PHVSDS:

Variable	B	se	β	P	N
1. BMISDS@ PHV	-.19	.35	-.12	.60	23
2. IGF-I	3.66	1.77	.42	.05	22
3. A4	2.99	1.48	.41	.06	22
4. DHEAS	-.23	.72	-.07	.75	22
5. E2	.70	.46	.32	.14	22
6. leptin	.27	3.21	.02	.93	14
7. SHBG	.45	1.61	.06	.78	22
8. testosterone	.36	.77	.10	.64	23
9. FAI	.12	.60	.05	.84	22
10. HbA1c	-.20	.10	-.41	.06	23
11. ins/kg	1.45	1.21	.25	.24	23
12. bone age	-.35	.25	-.30	.17	22

In a multivariable regression entering A4, IGF-I and HbA1c with PHVSDS as dependent variable (N=22*), both A4 and IGF-I are independently significant determinants, B = 2.98 ± 1.30, P = 0.04 with β = 0.43 and B = 3.46 ± 1.55, P = 0.04 with β = 0.42 respectively; P of the model = 0.02 and* R^2 *= 29.2%.*

6.6.2.4 Summary

In 'all boys', A4, IGF-I and testosterone levels at PHV are all positively and significantly correlated with PHVSDS (p = 0.004, p = 0.05, p = 0.04 respectively). In a multivariable model, A4 is significant adjusting for levels of IGF-I and there is a trend towards significance in the same model for IGF-I (P = 0.08). This model is significant, p = 0.004 and explains 20.5% of the variance. Higher levels of these hormones at PHV, each adjusting for the other, are related to a greater PHVSDS. In the control boys, in a multivariable model, testosterone levels at PHV are positively and significantly associated with PHVSDS in a model that explains 36.2% of the variance. In the T1D boys both A4 and IGF-I levels at PHV are independently and significantly related to

PHVSDS such that higher levels of these hormones at PHV are associated with a greater PHVSDS. This model explains 29.2% of the variance.

6.6.3 Girls

6.6.3.1 All Girls

Univariable regressions with PHVSDS:

Variable	B	se	β	P	N	P adj for disease
1. BMISDS @PHV	-.27	.11	-.33	.02	52	.03
2. IGF-I	-.53	1.07	-.07	.62	47	.43
3. A4	.68	.61	.18	.27	38	.41
4. DHEAS	-.23	.35	-.11	.52	38	.27
5. E2	.15	.41	.06	.72	46	.90
6. leptin	-.80	.56	-.26	.16	31	.49
7. SHBG	1.19	.90	.22	.19	38	.16
8. testosterone	.05	.49	.02	.92	47	.99
9. FAI	-.16	.48	-.06	.74	37	.56

In a multivariable regression entering BMISDS and 'disease' with PHVSDS as the dependent variable (N=52), *only BMISDS has a significant relationship with PHVSDS: B = - 0.27 ± 0.11, P = 0.02 with β = - 0.33, P of model = 0.02 and R^2 = 9.1%.*

6.6.3.2 Control Girls

Univariable regressions with PHVSDS:

Variable	B	se	β	P	N
1. BMISDS @PHV	-.15	.12	-.24	.21	30
2. IGF-I	-.78	1.36	-.11	.57	27
3. A4	.07	1.08	.02	.95	20
4. DHEAS	-.04	.56	-.02	.94	20
5. E2	-.04	.43	-.02	.94	26
6. leptin	-.38	.79	-.11	.64	20
7. SHBG	.35	1.19	.07	.77	20
8. testosterone	.14	.56	.05	.80	27
9. FAI	.23	.57	.09	.70	20

There is no significant relationship between any of these variables at PHV and PHVSDS in the control girls.

6.6.3.3 T1D Girls

Univariable regressions with PHVSDS:

Variable	B	se	β	P	N
1. BMISDS @ PHV	-.52	.23	-.45	.04	22
2. IGF-I	-1.02	1.87	-.13	.59	20
3. A4	.87	1.02	.21	.41	18
4. DHEAS	-.67	.55	-.29	.24	18
5. E2	.34	1.01	.08	.74	20
6. leptin	-.77	1.60	-.16	.64	11
7. SHBG	2.10	1.37	.36	.15	18
8. testosterone	-.25	.92	-.06	.79	20
9. FAI	-.98	.87	-.28	.28	17
10. HbA1c @ PHV	-.14	.13	-.24	.29	22
11. ins/kg @ PHV	.39	.72	.12	.59	22
12. bone age @ PHV	-.50	.22	-.48	.03	20

Only BMISDS and bone age at PHV have a significant relationship with PHVSDS in these T1D girls: r = - 0.45, P = 0.04 and r = - 0.48, P = 0.03.

In a multivariable regression entering BMISDS and bone age at PHV with PHVSDS as the dependent variable (N=20) *only bone age is a significant determinant: B = - 0.50 ± 0.22, P = 0.03 with β = - 0.48 and P of the model = 0.03 with R^2 = 18.4%.*

6.6.3.4 Summary

In 'all girls', BMISDS at the time of PHV was the only variable studied that had any association with PHVSDS. This was negative and significant (P = 0.02) thus girls who had a greater BMISDS by the time of PHV would have a diminished PHVSDS. This only explained 9.1% of the variance in PHVSDS and so there are obviously other as yet unidentified variables involved. Nothing of interest was found in the control girls but it should be remembered that the age

at occurrence of PHV has already been adjusted for in the calculation of PHVSDS. In the T1D girls an advanced bone age has previously been observed at the time of PHV and although BMISDS on its own was negatively and significantly related to PHVSDS it was not significant in this model with bone age as a covariate. Bone age was a significant (P = 0.03) negative determinant of PHVSDS, so those girls who have a more advanced bone age at PHV have a lower PHVSDS.

6.7 PHVSDS and hormones/biological variables averaged from Stage 2 to PHV

6.7.1 Question:

What effect, if any, do the various hormones (averaged from stage 2 to PHV) or BMISDS at stage 2 have an on PHVSDS? Do any associations differ between T1D and control children?

6.7.2 Boys

6.7.2.1 All Boys

Univariable regressions with PHVSDS:

Variable	B	se	β	P	N	P adj for disease
1. BMISDS @ G2	-.24	.18	-.20	.19	46	.27
2. IGF-I	.90	1.76	.08	.61	46	.74
3. A4	1.49	.87	.25	.09	45	.11
4. DHEAS	-.68	.51	-.20	.19	45	.12
5. E2	Not available for controls					
6. leptin	-.84	.98	-.15	.40	36	.81
7. SHBG	2.95	1.21	.35	.02	45	.005
8. testosterone	.42	.56	.11	.46	46	.55
9. FAI	-.03	.45	-.01	.95	45	.78

In a multivariable regression entering A4 and 'disease' with PHVSDS as the dependent variable (N=45), *there is only a trend towards significance with A4*

averaged levels from G2-PHV and PHVSDS: B = 1.49 ± 0.87, P = 0.09, with β = 0.25 and R^2 = 4.3%.

6.7.2.2 Control Boys

Univariable Regressions with PHVSDS:

Variable	B	se	β	P	N
1. BMISDS @G2	-.28	.27	-.22	.31	23
2. IGF-I	-1.83	2.95	-.13	.54	23
3. A4	1.11	1.07	.23	.31	22
4. DHEAS	-.87	.80	-.24	.29	22
5. E2	Not available				
6. leptin	-1.32	1.65	-.19	.43	20
7. SHBG	5.33	1.62	.59	.004	22
8. testosterone	.39	1.04	.08	.71	23
9. FAI	-.67	.96	-.15	.50	22

None of the hormones averaged over the period from the start of puberty to PHV nor BMISDS at the start of puberty have been found to play a significant role in PHVSDS in the control boys.

6.7.2.3 T1D Boys

Univariable regressions with PHVSDS:

Variable	B	se	β	P	N
1. BMISDS@ G2	-.13	.31	-.09	.68	23
2. IGF-I	2.45	2.22	.23	.28	23
3. A4	2.62	1.72	.32	.14	23
4. DHEAS	-.79	.68	-.25	.26	23
5. E2	.88	.48	.37	.08	23
6. leptin	1.49	2.01	.19	.47	16
7. SHBG	1.66	1.84	.19	.38	23
8. testosterone	.32	.67	.11	.63	23
9. FAI	.07	.51	.03	.90	23
10. HbA1c	-.31	.13	-.47	.02	23
11. ins/kg	.64	1.49	.09	.67	23
12. bone age @G2	-.25	.16	-.36	.14	18

In a multivariable regression entering HbA1c and E2 with PHVSDS as the dependent variable (N=23), *both HbA1c (B = - 0.34 ± 0.12, P = 0.009 with β = -*

0.51) and E2 (B = 1.00 ± 0.42, P 0.03 with β = 0.42), P of the model = 0.0007 and R^2 = 33.4%.

6.7.2.4 Summary

In 'all boys' and control boys, there were no hormones averaged from G2 to PHV that appear to play any significant role in PHVSDS. There was a modest trend with A4 in 'all boys'. In T1D boys both HbA1c and E2 averaged over the time were independently associated with PHVSDS. Adjusting for levels of E2, higher HbA1c (poorer control) during this time is associated with a lower PHVSDS and higher levels of E2 (adjusting for HbA1c) with a higher PHVSDS.

6.7.3 Girls

6.7.3.1 All Girls

Univariable regressions with PHVSDS:

Variable	B	se	β	P	N	P adj for disease
1. BMISDS@B2	-.27	.12	-.31	.02	52	.04
2. IGF-I	-.54	.99	-.08	.59	47	.45
3. A4	.87	.66	.21	.19	39	.22
4. DHEAS	-.10	.35	-.05	.78	39	.59
5. E2	.52	.45	.17	.26	46	.32
6. Leptin	-.77	.54	-.25	.16	32	.50
7. SHBG	1.30	.99	.21	.20	39	.18
8. testosterone	.46	.54	.13	.40	47	.45
9. FAI	.02	.51	.007	.97	39	.94

BMISDS at B2 is significantly (P = 0.02), albeit weakly (r = - 0.31) associated with PHVSDS. None of the hormones averaged from the start of puberty to PHV are related to PHVSDS

In a multivariable regression entering BMISDS and 'disease' with PHVSDS as the dependent variable (N=52), only *BMISDS is significant: B = - 0.27 ± 0.12, P = 0.02 with β = - 0.31 and R^2 = 8%.*

6.7.3.2.Control Girls

None of the variables considered in the univariable correlation matrix with PHVSDS had a correlation with a P value less than 0.2 (it was surprising that even BMISDS had a modest association that was not significant: r = - 0.23, P = 0.21). In the multivariable regression no significant associations were found although it should be remembered that the calculation of PHVSDS in itself includes an allowance for age of occurrence of PHV.

6.7.3.3 T1D Girls:

Univariable regressions with PHVSDS:

Variable	B	se	β	P	N
1. BMISDS@B2	-.50	.25	-.43	.05	21
2. IGF-I	-1.36	1.44	-.21	.36	21
3. A4	1.25	1.19	.24	.31	20
4. DHEAS	-.47	.55	.20	.41	20
5. E2	1.66	1.40	.26	.25	21
6. leptin	-.73	1.45	-.16	.63	12
7. SHBG	2.12	1.38	.34	.14	20
8. testosterone	.10	1.14	.02	.93	21
9. FAI	-.64	.93	-.16	.50	20
10. HbA1c	-.16	.14	-.25	.27	22
11. ins/kg	.32	.68	.11	.64	22
12. bone age @ B2	-.49	.21	-.52	.03	17

In a multivariable regression entering BMISDS @ B2 and bone age @ B2 with PHVSDS as the dependent variable (N=17), *BMISDS@B2 is a significant determinant of PHVSDS in the T1D girls: B = - 0.68 ± 0.26, P = 0.02 with β = - 0.43 and R^2 = 26.4%.*

6.7.3.4 Summary

BMISDS at B2 is negatively related to PHVSDS in 'all girls' indicating that relatively thinner girls at the start of puberty will have a greater PHVSDS. This only accounts for 8% of the variation however so there are still a lot of unidentified determinants. No associations with PHVSDS and any of the

variables considered here were observed for control girls although again it should be remembered that the calculation of PHVSDS includes an adjustment for the age of PHV occurrence. In T1D girls, only BMISDS ($r = -0.43$, $P = 0.05$) and bone age at B2 were related ($r = -0.52$, $P = 0.03$) to PHVSDS and in a multivariable model only BMISDS retained a significant influence on PHVSDS explaining 26.4% of the variance.

6.8 Bone Age at Puberty Onset in T1D Children

6.8.1 Question

Is it possible to identify the variables at the start of puberty that might affect bone age in the T1D children?

6.8.2 TID Boys

In this dataset, we have observed that T1D boys enter puberty a year later than control boys with a bone age that was less than their chronological age although not statistically significant (chapter 3, section 3.3.3).

Univariable regressions with bone age at G2:

Variable	B	se	β	P	N
1. BMISDS@G2	-.11	.48	-.06	.83	18
2. IGF-I	1.65	2.47	.17	.52	17
3. A4	-3.47	2.57	-.35	.20	15
4. DHEAS	-.05	1.41	-.01	.97	15
5. E2	.56	.75	.20	.47	16
6. leptin	.32	3.81	.03	.94	10
7. SHBG	2.78	3.86	.20	.48	15
8. testosterone	.12	.89	.04	.89	17
9. FAI	-.26	.88	-.08	.77	15
10. HbA1c	.52	.22	.52	.03	18
11. ins/kg	.66	2.02	.08	.75	18
12. CA@B2	.93	.28	.64	.004	18

A multivariable regression entering HbA1c and CA at G2 with bone age at G2 as the dependent variable (N=18) results in only *chronological age as significant determinant of bone age at start of puberty: B = 0.93 ± 0.28 with β = 0.64, P = 0.004 with R^2 = 36.9%.*

6.8.3 Summary

There is no association with any of the hormones studied at the start of puberty with the bone age at this time in the T1D boys. It is only chronological age and HbA1c that are significant. When entered into a model together it is only

chronological age that appears to exert a significant effect. Thus in T1D boys a greater chronological age at G2 is associated with a more advanced bone age.

6.8.4 TID Girls

Univariable regressions with bone age at B2:

Variable	B	se	β	P	N
1. BMISDS@B2	.56	.31	.43	.09	17
2. IGF-I	1.96	1.59	.30	.24	17
3. A4	.43	1.25	.09	.74	17
4. DHEAS	.78	.61	.31	.22	17
5. E2	2.28	1.64	.34	.18	17
6. leptin	2.58	1.65	.62	.19	6
7. SHBG	-2.25	1.67	-.33	.20	17
8. testosterone	1.39	1.65	.21	.42	17
9. FAI	1.46	1.11	.32	.21	17
10. HbA1c	-.03	.18	-.04	.89	17
11. ins/kg	.33	1.05	.08	.76	17
12. CA@ B2	.65	.43	.36	.16	17

A multivariable regression entering chronological age and BMISDS at B2 with bone age at B2 as the dependent variable (N=17) results in *BMISDS being a significant determinant B = 0.61 ± 0.29 with β = 0.47, P = 0.05 adjusting for chronological age (B = 0.73 ± 0.39 with β = 0.40, P = .08) with P of the model = 0.05 and R^2 = 25.1%.*

6.8.5 Summary

BMISDS at the start of puberty is positively correlated with bone age at pubertal onset in the T1D girls, but is not quite significant in this sample, P = 0.09. In the multivariable model including chronological age and BMISDS, BMISDS remains significant adjusting for chronological age. Thus a relatively greater BMI at the onset of puberty, adjusting for chronological age at this time, is associated with a more advanced bone age at puberty onset.

Chapter 7. Conclusions

The current data collected over many years has provided some fascinating insights into the variation and regulation of the timing and endocrine control of puberty in children with and without T1D. A summary of the observations are as follows:

7.1 Timing and variation in pubertal growth in T1D children

- Using contemporary controls and population reference comparisons, the current study indicates that there are subtle anomalies in the growth of children with T1D.
- The T1D boys had a relatively late pubertal onset but essentially normal growth while the girls had a slightly earlier age of pubertal onset (compared to controls) with an advanced bone age and a subsequent reduced PHV.

7.2 BMI, Body Composition

- This longitudinal data has contributed to the understanding of BMI, FM and FFM changes during puberty in normal children and those with T1D and the possible role of leptin.
- BMI increased in all the children over this pubertal time with the T1D children of both sexes having greater BMI levels than controls.
- The sexual dimorphism in this BMI was expressed as a greater acquisition of FFM in the boys and FM in the girls.
- Leptin was higher in the T1D cohort and higher at all levels of fat mass. This may represent a degree of leptin resistance in both sexes but it was

only the T1D girls who gained more FM than controls whereas the T1D boys had higher gains in FFM but still had higher leptin levels.

7.3 Hormonal Changes during Puberty

- This unique longitudinal study provides insights relating to hormonal changes during normal puberty and their relationship to PHV, puberty onset and menarche. The data clearly define hormonal abnormalities in IGF-I, DHEAS, leptin and testosterone levels in T1D subjects compared with controls and the degree to which these may be explained by peripheral vs portal insulin administration.
- In an effort to find explanations for the observed differences between the T1D and control subjects this comparative study has tried to establish important determinants of the timing and the extent of pubertal variance in growth and body composition.

7.4 Timing of Puberty and Pubertal Growth in Control Boys

- Curiously the only correlate with timing of puberty was DHEAS, lower levels predicting earlier puberty, this is the converse of the adrenarche/puberty hypothesis. The variance was only 18.4% and it was a relatively small sample.
- The association between testosterone and the duration from G2-PHV was interesting, higher testosterone levels at puberty onset predicted a shorter duration and explained 43.3% of the variance.
- HtSDS change between G2 and PHV was strongly predicted by duration G2-PHV. Associations were also observed with leptin and age at start of puberty. Lower leptin was associated with greater relative height gain. A

later age at start of puberty predictably had a negative effect on subsequent height gain.

- Analysis of PHVSDS and the hormones at PHV revealed that only testosterone was significant. Calculation of PHVSDS already allows for the effects of age at PHV.

7.5 Timing of Puberty and Pubertal Growth in Control Girls

- Timing of puberty onset in girls was strongly related to BMISDS and E2 levels at the start of puberty (explaining 61% of the variance).
- A longer duration from B2 to PHV was also explained by a greater BMISDS.
- These observations raise issues about the accurate timing of pubertal onset and whether an earlier B2 and modest elevations in E2 may precede the true onset of puberty in overweight girls. Recent discrepant observations in the literature associate earlier B2 with overweight but there is no similar trend for an earlier menarche.
- A longer duration was also associated with lower levels of IGF-I. Generally children with a higher BMI have higher IGF-I levels but it would appear that these determinants are independent.
- Height gain was strongly predicted by the duration from B2 to PHV and an earlier age of puberty onset was associated with a greater height gain.
- PHVSDS was negatively associated with BMISDS consistent with the hypothesis that overweight and early puberty may lead to the blunting of the pubertal growth spurt.
- None of the hormonal variables explained the variation in PHVSDS.

7.6 Timing of Puberty and Pubertal Growth in T1D Boys

- Pubertal growth in T1D boys has been observed to be remarkably normal (chapter 3) despite low levels of IGF-I, testosterone and DHEAS.
- The delay in pubertal onset was related to the presence of 'disease' and was not explained by hormone levels. The positive association with bone age is suggestive of a slowing in the growth tempo.
- The duration from G2 to PHV was shorter in T1D boys relative to controls as seen in chapter 3. A longer duration was associated with lower testosterone levels at the start of puberty. Perhaps the 'disease' effect is partly reflected in a trend towards higher HbA1c and later pubertal onset.
- Height gain from G2 to PHV was, as in control boys, strongly related to a greater duration G2-PHV, however, a high HbA1c was independently associated with a reduced height gain.
- PHVSDS determinants (other than age which is inherent in the calculation) varied depending on whether hormones at PHV or hormones from G2 to PHV were considered. A4 and IGF-I levels at PHV were positive determinants whereas high HbA1c values in the time from G2-PHV were associated with a diminished PHVSDS.
- Overall the presence of T1D speeds up the growth tempo and the associated low testosterone, A4 and IGF-I levels with high HbA1c have negative effects on pubertal growth but this is marginal. An interesting speculation is whether the high GH levels may, through direct effects, be protective of overall growth in boys with T1D and could also explain the increased FFM described in chapter 4.

7.7 Timing of Puberty and Pubertal Growth in T1D Girls

- T1D girls entered puberty with an advanced bone age although their chronological age was similar to controls.
- Interestingly the onset of puberty was not related to BMISDS as it was in the control girls but a higher HbA1c at the start of puberty was associated with a later age of puberty onset.
- Bone age at the start of puberty was positively related to BMISDS.
- Duration of B2-PHV was no different in T1D girls and controls as seen in chapter 3. This duration was negatively associated with levels of IGF-I at the start of puberty in both cohorts. Although one might have predicted that lower IGF-I levels in the T1D girls may slow pubertal progression, significant IGF-I differences between the cohorts don't usually show up until later in puberty.
- Height gain from B2-PHV was strongly related to both age at onset and duration B2-PHV but there was an indication that a high HbA1c and low E2 levels might also contribute to poor height gain.
- PHVSDS was negatively associated with BMISDS and bone age; such that a higher BMISDS was associated with a lower PHVSDS as was an advanced bone age.
- Overall it would appear that BMISDS, HbA1c and bone age at the onset of puberty are more important than hormonal changes in the blunting of the pubertal growth spurt in T1D girls.

7.8 Summary and highlights from the Conclusions

In this cohort of T1D boys, the onset of puberty was later than in controls, but pubertal growth appeared to be normal in spite of lower levels of IGF-I, testosterone, A4 and DHEAS. There were no hormonal factors identified that influenced this delay in onset but rather it appears to be inherent in the disease process itself.

Puberty duration was longer in control boys and was associated with lower levels of testosterone. Poorer glycaemic control (higher HbA1c) at the start of puberty in the diabetic boys, perhaps as part of the disease effect, also played a role in shortening pubertal duration. The intensity of the growth spurt (as reflected by PHVSDS) was related to testosterone levels in the control boys and in the diabetic boys was positively associated with levels of IGF-I and A4 but adversely affected by levels of HbA1c. A reduced relative pubertal height gain was associated with higher values of HbA1c.

Although the pubertal growth tempo was accelerated in T1D boys compared to the controls, the lower levels of IGF-I, A4, DHEAS and testosterone appears to play a minimal role.

The T1D girls started puberty at a similar age to the control girls but with an advanced bone age. Unlike the boys, pubertal onset in the diabetic girls was influenced by glycaemic control in that a higher HbA1c was associated with a delayed pubertal onset. There appeared to be a subsequent slowing down in pubertal tempo and the diabetic girls took significantly longer to get to the end of puberty (stage 5). The well known positive association of BMI and age at puberty onset was observed in the control girls but was not present in the

diabetic girls. A greater BMI at the start of puberty in the diabetic girls was associated with a more advanced bone age at this time.

A greater BMI was also associated with a longer pubertal duration in control girls and in both cohorts a lower IGF-I level at the start of puberty played a role in extending the duration from the onset of puberty until PHV. Low oestrogen levels in the diabetic girls led to less relative height gain during puberty but as in the boys, poorer glycaemic control was also a contributing factor. The diabetic girls had a diminished growth spurt that was related to both bone age and BMISDS at the time of PHV however none of the hormones studied were identified as factors in the growth spurt in the girls.

Although it is possible that peripheral insulinaemia may be associated with increased leptin secretion, it remains an enigma as to why the leptin levels in both sexes were higher in the T1D cohort compared to the controls but it is only the girls who had greater fat mass while the T1D boys gained more fat free mass.

Thus, these observations reveal a number of sex differences as well as subtle abnormalities that exist in the pubertal growth and development of children with T1D.

7.9 Strengths and Weaknesses of this study

Strengths:

- Longitudinal
- Contemporary controls with comparable protocols
- Study comprises both auxology (including experienced observer-assessed puberty ratings) and biochemistry
- Seen frequently throughout puberty (3 monthly for diabetic cohort and 6 monthly for controls)
- Largely centralised assays
- Small number of observers trained initially together
- Long duration of follow up

Weaknesses:

- Relatively small sample sizes
- Not a prospective matched design
- Observational studies, difficult to prove causality
- Not followed to final height
- No bone age in controls
- No information on menstrual cycle in the girls
- No measurement of binding proteins for IGF-I

Despite the caveats, the data here provide a unique window into variations in growth, body composition and hormonal changes in young people with T1D vs controls. Larger longitudinal studies with the principal aim of understanding the effect of hormonal/metabolic variation on the timing and pubertal variation in

growth and body composition in T1D would add additional insights and would be an interesting future study.

Chapter 8. Appendix

8.1 Lower limits of Assays:

Where a value for an assay was given as < the lower limit of that assay, a number half way between that value and 0 has been used. When the value was given as equal to the lower limit than that was recorded as reported, thus differentiating between testosterone=0.8 and testosterone=<0.8 (recorded as 0.4).

As more sensitive assays have been developed, the lower limits of the assays have varied over the years that the data was collected, ie testosterone has changed from <0.8 to <0.4.

8.2 Menarcheal Age:

For most of the T1D girls this was known to the day, for the few where it was not, the month was known and in those cases I have used the 15^{th} of that month.

For the control girls the data was recorded as '0' for no menarche and '1' for menarche. To calculate the age of menarche I have used the average of the two ages on these two occasions provided the interval between them was no longer than six months. In those cases where it was greater than six months I have not included these girls in the assessment of menarcheal age.

8.3 Puberty Stage at Menarche in the Control Study:

Since the exact time of menarche was unknown and even if it could ever be known, the chance of knowing the puberty stage at that moment would be slight. Therefore the puberty stage assigned at the time of menarche was the one the

girl was in at both time '0' and time'1' if they were the same but if the girl had progressed in the 6 months then the stage at time '0' was recorded.

8.4 Choice of hormone values

Hormones in a particular puberty stage where a child appears more than once in that stage have been averaged for that stage. This was regarded as the most representative of hormone levels at that point. There will be an innate variability and or diurnal variation that the averaged value will smooth out and was felt to be more representative of the 'true' value. We can never really know when the transition from one stage to another really occurs.

8.5 Puberty ratings

Female puberty ratings in both controls and T1D were probably more consistent than males since one female observer who had been trained by the same investigator did each study. In the controls there had been 2 male investigators who again had trained with the same original investigator while in the diabetic clinic there had been an assortment of male clinicians over the years although they were trained by one of the control investigators who himself trained at the same original centre.

8.6 Reliability

As mentioned in the text I have used the TEM as the measure of precision as I was able to find values in the literature against which to compare. Below are the raw data for both skinfolds and height test-retest

8.6.1 Skinfold Measurements

	Biceps				Triceps				Subscapular				Suprailiac			
child	time 1	time 2	diff (1-2)	diff^2	time 1	time 2	diff (1-2)	diff^2	time 1	time 2	diff (1-2)	diff^2	time 1	time 2	diff (1-2)	diff^2
1	7.4	8.0	-0.6	0.36	12.2	12.4	-0.2	0.04	5.6	5.5	0.1	0.01	5.4	6.0	-0.6	0.36
2	9.1	8.8	0.3	0.09	16.6	16.0	0.6	0.36	7.2	7.0	0.2	0.04	9.2	9.1	0.1	0.01
3	8.1	7.8	0.3	0.09	14.2	14.1	0.1	0.01	8.4	8.3	0.1	0.01	8.1	8.1	0.0	0.00
4	9.8	10.2	-0.4	0.16	15.2	15.4	-0.2	0.04	8.8	8.6	0.2	0.04	9.8	11.4	-1.6	2.56
5	5.0	6.2	-1.2	1.44	7.6	8.8	-1.2	1.44	4.8	5.3	-0.5	0.25	2.8	3.1	-0.3	0.09
6	6.2	6.2	0.0	0.00	9.7	8.4	1.3	1.69	5.8	5.7	0.1	0.01	3.0	3.2	-0.2	0.04
7	3.4	3.8	-0.4	0.16	7.4	7.0	0.4	0.16	5.1	5.0	0.1	0.01	4.2	4.6	-0.4	0.16
8	3.4	3.8	-0.4	0.16	5.4	4.9	0.5	0.25	4.4	4.4	0.0	0.00	3.6	3.6	0.0	0.00
9	2.5	2.5	0.0	0.00	4.6	4.6	0.0	0.00	3.5	3.4	0.1	0.01	2.4	2.4	0.0	0.00
10	10.	10.0	0.6	0.36	17.4	16.0	1.4	1.96	10.8	10.2	0.6	0.36	13.9	13.4	0.5	0.25
11	7.0	6.6	0.4	0.16	12.0	10.4	1.6	2.56	6.6	6.2	0.4	0.16	6.8	6.7	0.1	0.01
12	10.	12.2	-1.8	3.24	16.4	17.2	-0.8	0.64	11.2	11.2	0.0	0.00	12.8	13.0	-0.2	0.04
13	4.2	3.8	0.4	0.16	11.2	11.2	0.0	0.00	5.2	4.8	0.4	0.16	4.4	4.4	0.0	0.00
14	4.1	4.0	0.1	0.01	7.0	6.6	0.4	0.16	5.2	5.2	0.0	0.00	5.1	5.4	-0.3	0.09
15	7.8	8.2	-0.4	0.16	13.0	13.1	-0.1	0.01	6.1	6.3	-0.2	0.04	6.1	6.1	0.0	0.00
16	4.6	5.2	-0.6	0.36	9.4	8.6	0.8	0.64	4.0	4.1	-0.1	0.01	3.3	3.3	0.0	0.00
17	10.	10.4	-0.1	0.01	17.2	17.0	0.2	0.04	10.2	10.0	0.2	0.04	10.4	10.0	0.4	0.16
18	8.0	9.4	-1.4	1.96	16.0	15.0	1.0	1.00	8.1	7.0	1.1	1.21	9.4	9.4	0.0	0.00
19	7.1	6.4	0.7	0.49	12.1	11.6	0.5	0.25	5.2	5.2	0.0	0.00	4.4	4.1	0.3	0.09
20	2.4	2.3	0.1	0.01	7.3	6.6	0.7	0.49	4.3	4.4	-0.1	0.01	3.4	3.0	0.4	0.16
21	10.	10.4	-0.2	0.04	14.4	13.9	0.5	0.25	12.0	10.8	1.2	1.44	8.8	8.9	-0.1	0.01
22	7.2	8.4	-1.2	1.44	12.6	12.6	0.0	0.00	8.0	8.2	-0.2	0.04	5.6	6.0	-0.4	0.16
23	9.4	9.4	0.0	0.00	14.2	14.0	0.2	0.04	8.0	7.9	0.1	0.01	7.4	8.5	-1.1	1.21
24	9.4	9.4	0.0	0.00	12.0	12.0	0.0	0.00	7.8	7.8	0.0	0.00	6.6	7.0	-0.4	0.16
25	5.9	6.1	-0.2	0.04	12.0	13.0	-1.0	1.00	5.8	5.4	0.4	0.16	4.2	4.7	-0.5	0.25
26	4.4	4.2	0.2	0.04	9.0	9.8	-0.8	0.64	5.2	5.0	0.2	0.04	3.8	4.0	-0.2	0.04
27	12.	12.0	0.2	0.04	17.2	17.2	0.0	0.00	9.4	10.4	-1.0	1.00	14.2	15.2	-1.0	1.00
28	7.4	7.8	-0.4	0.16	16.0	15.9	0.1	0.01	8.8	7.6	1.2	1.44	5.8	6.4	-0.6	0.36
29	4.2	4.6	-0.4	0.16	9.8	9.0	0.8	0.64	5.2	5.0	0.2	0.04	4.2	3.8	0.4	0.16
30	4.8	4.4	0.4	0.16	7.1	8.0	-0.9	0.81	4.9	4.8	0.1	0.01	3.3	3.9	-0.6	0.36
31	4.7	4.3	0.4	0.16	9.9	10.0	-0.1	0.01	4.6	4.7	-0.1	0.01	3.0	3.1	-0.1	0.01
32	5.2	4.7	0.5	0.25	10.4	9.6	0.8	0.64	4.0	4.2	-0.2	0.04	2.8	3.0	-0.2	0.04
33	5.1	5.0	0.1	0.01	10.4	10.2	0.2	0.04	5.4	5.4	0.0	0.00	5.4	5.5	-0.1	0.01
34	7.0	7.0	0.0	0.00	13.2	14.2	-1.0	1.00	6.1	6.2	-0.1	0.01	5.6	5.4	0.2	0.04
35	5.3	5.2	0.1	0.01	12.0	11.6	0.4	0.16	5.8	5.1	0.7	0.49	4.9	5.4	-0.5	0.25
36	5.3	5.6	-0.3	0.09	12.0	11.0	1.0	1.00	5.8	5.4	0.4	0.16	4.8	4.9	-0.1	0.01

	$\sum d^2$	$\sum d^2/2N$	TEM	1.96*TEM
Bicep	11.98	.166	.41	.80
Tricep	17.98	.250	.50	.98
Subsp	7.26	.101	.32	.62
Spril	8.09	.112	.34	.66

8.6.2 Height

N	Time1	Time2	Diff (T1-T2)	D^2
1	137.4	137.5	-0.1	0.010
2	145.2	145.0	0.2	0.040
3	125.0	124.9	0.1	0.010
4	145.7	145.9	-0.2	0.040
5	142.4	142.5	-0.1	0.010
6	158.8	159.3	-0.5	0.250
7	118.5	118.2	0.3	0.090
8	148.5	148.8	-0.3	0.090
9	143.3	143.5	-0.2	0.040
10	145.6	145.8	-0.2	0.040
11	137.8	137.5	0.3	0.090
12	153.5	153.2	0.3	0.090
13	159.3	159.2	0.1	0.010
14	150.5	150.4	0.1	0.010
15	146.4	146.2	0.2	0.040
16	142.4	142.2	0.2	0.040
17	147.7	147.8	-0.1	0.010
18	161.8	161.7	0.1	0.010
19	147.7	147.6	0.1	0.010
20	163.6	163.8	-0.2	0.040
21	158.7	158.6	0.1	0.010
22	174.3	174.1	0.2	0.040
23	125.1	125.0	0.1	0.010
24	105.1	105.2	-0.1	0.010
25	137.2	137.3	-0.1	0.010
26	152.7	153.1	-0.4	0.160
27	140.9	140.8	0.1	0.010
28	143.7	143.5	0.2	0.040
29	151.1	151.2	-0.1	0.010
30	146.7	146.6	0.1	0.010
31	148.8	148.8	0.0	0.000
32	145.6	145.7	-0.1	0.010
33	155.0	154.8	0.2	0.040
34	158.7	158.5	0.2	0.040

Summaries from above height data:

$\sum d^2$	$\sum d^2/2N$	**TEM**	**1.96*TEM**
1.37	.02	.14	.28

8.7 Comment on the ascertainment of the age of onset of puberty:

A number of children in both the T1D and control cohort were seen for the first time either in stage 2 or had a stage 2 rating that was more than 6 months after a previous rating of a stage 1. This situation occurred since several children were not seen due to missed appointments or inadvertently not examined (in the case of some of those with T1D) until they had begun puberty and were found to be in stage 2. There were also several control children who appeared to go from stage 1 to stage 3 between their 6-month visits, whether this meant they moved rapidly through this pubertal time or whether it relates to the subjectiveness of the rating is unknown. Although there was no statistical difference in the mean age of pubertal onset in any group whether all the data was included or only the data of those where a more rigorous approach was applied (including only those where a previous stage 1 within a 6 month period was available), in order to arrive at as precise an estimate of age of pubertal onset in each cohort as possible, only those children with the rigorous approach have been included for this analysis.

This table shows the differences in the ages of pubertal onset if all the data had been included (white columns) or just those where the age was more rigorously determined (grey columns).

	GIRLS				BOYS			
	T1D		Controls		T1D		Controls	
N	23	16	53	45	26	21	43	42
Mean	10.91	11.05	11.19	11.37	12.24	12.32	11.28	11.28
sd	0.87	0.60	1.19	1.00	0.99	0.94	0.78	0.78
Median	10.83	10.92	11.18	11.43	12.12	12.13	11.30	11.28
Min	8.54	9.95	9.44	10.01	10.83	11.05	10.09	10.09
Max	12.48	11.87	14.08	13.86	14.67	14.67	13.77	13.77

8.8 HbA_1 and HbA_{1c} Methodology Changes

Prior to 1992 the Department of Clinical Biochemistry at the John Radcliffe Hospital used an electrophoretic (electro endosmosis) method for the estimation of glycated haemoglobin (Ciba Corning Diagnostics, Halstead UK). This method separates HbA_0 from HbA_1 and HbF which migrate together as a single peak 'HbA_1' and is expressed as a percentage of the total haemoglobin.

In February 1992 this method was replaced by the Diamat (Bio-Rad Laboratories, Hemel Hempstead, UK) an automated high performance liquid chromatography system (HPLC). This machine measures five fractions separately: HbA_0, HbA_{1a}, HbA_{1b}, HbA_{1c}, and HbF and thus the estimation of HbA_{1c} or 'true' HbA_1 can be made (by summing HbA_{1a}, HbA_{1b} and HbA_{1c}) without the contribution of HbF.

The Department of Clinical Biochemistry provided an equation to allow conversion of the earlier Corning electrophoresis HbA1 and the later Diamat HbA_{1c} by HPLC: Derived HbA_{1c} = (Corning HbA1 x 0.86) - 0.4. Where:0.4 = the intercept and represents an average value for the contribution of HbF and 0.86 = the slope and allows the conversion of 'true' HbA_1 (HbA_{1a+b+c}) to HbA_{1c}

HbA1c normal range was 4.3-6.1% with the intra-assay imprecision being 1.9 and 2.2% at HbA1c levels of 6.9 and 11.5% respectively, and inter-assay imprecision was 2.7 and 2.3% at HbA1c levels of 7.0 and 11.6% respectively.

8.9 Other Laboratory Assays

Serum samples were separated, stored at -20^{O}C and subsequently used for the measurement of testosterone, oestradiol, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), androstenedione (A4), insulin-like growth factor-I (IGF-I) and thyroid hormones.

1. Serum IGF-I concentrations were determined by radioimmunoassay (RIA) after acid-ethanol extraction (Morrell et al. 1989). The intra-assay imprecisions were 5.2 and 4.8% at 27.5 and 220 ng/mL respectively. The

inter-assay imprecisions were 12.7 and 10.6% at 77 and 242 ng/mL respectively.

2. Free T4 was measured by a two-step, back titration method previously validated against an equilibrium dialysis method (Dunger et al. 1990). The inter-assay imprecision was 8.6%.

3. Serum oestradiol was determined using the double antibody Diagnostics Products Corp kit (Llanberis, Wales, UK). The inter-assay imprecision was <10% at the three concentrations tested (approximately 150, 500 and 1000 ρmol/L).

4. Serum testosterone and androstenedione were measured using an in-house RIA after ether extraction. The assays use an iodinated tracer and a dextran-coated charcoal separation-step. Between assay imprecision was <10% for both assays (Wathen et al. 1987).

5. SHBG was measured using a ^{3}H saturation assay (Fattah and Chard 1981). Inter-assay variation was <8% at the concentrations (20,40 and 70 nmol/L) tested.

6. DHEAS was determined with in-house reagents. The assay required pre-dilution of the sample (20-fold) with assay buffer followed by RIA with an ^{125}I tracer. Inter-assay imprecision was <10% at the concentrations (2, 10 and 20 μmol/L) tested (Wathen et al. 1987).

7. Serum leptin was measured by RIA (Linco Co., St Charles, MO, USA). All samples from a given individual were included in the same assay. The detection limit of the assay was 0.5 ng/mL (manufacturer's data). None of the sample values was less than 1.0ng/mL; therefore, there were no undetectable values. Intra- assay imprecisions were 5.7 and 6.7% at 2.5

and 12.5ng/ml respectively. Inter-assay imprecisions were 6.6 and 6.8% at 2.5 and 12.5ng/ml respectively.

Three hormones (testosterone, oestradiol and free T4) were not analysed in the same laboratory for both cohorts. The samples for the T1D subjects were analysed in the Department of Biochemistry at the John Radcliffe Hospital, Oxford whereas those for the control children were analysed in the Department of Clinical Biochemistry at St Bartholomew's Hospital, London. The assay methods previously described in the Appendix refer to those undertaken in the latter for the control group; the details below refer to the assays done in Oxford:

1. Testosterone was measured by an extraction method similar to that used for the control group until the mid 90's with an inter-assay imprecision <10%. Subsequent testosterone measurements were undertaken using a direct RIA kit obtained from Medgenix Diagnostics Ltd. The latter assay used an iodinated tracer that did not require extraction due to the high specificity of the coated antibodies. The intra-assay imprecision was 4 and 4.7% at 2.6 and 19.78nmol/l respectively and the inter-assay imprecision was 8.3 and 8.1% at 1.67 and 15.55nmol/l respectively.

It is difficult retrospectively to assess assay comparability between the two laboratory assays during this time period and whether potential differences may have invalidated the observed between group differences. Although Medgenix had better precision, there was little difference in method bias between the extraction RIA methods and the Medgenix assay and thus the results from the two labs would be very comparable (Dr Les Perry, London, personal communication). In addition, the reference intervals quoted were similar in both

laboratories, ie 9-35nmol/l from London and 9-42nmol/l from Oxford for adult males (personal communication from Dr Les Perry, London and Dr Tim James, Oxford). Therefore any differences between the methods can be considered modest compared to the observed differences in testosterone levels between the two cohorts of children.

2. Oestradiol was measured using a competitive radioimmunoassay utilising a precipitating second antibody and centrifugation, the kit was from Immuno Diagnostic Systems Ltd, and the principle of the method was similar to that used in London. Inter-assay imprecision was <10%. From January 1996, an automated competitive enzyme immunoassay method was used, Bayer Immuno 1 System, inter-assay imprecision <10%. Again it is difficult to ascertain retrospectively whether there were significant differences between the methods but both laboratories participated in external quality assessment schemes (EQA), which are designed to identify significant between laboratory differences. Neither laboratory reported any significant EQA problems and differences between cohorts are probably physiological.

3. FT4 was measured by a competitive analogue RIA from Amersham International until 1994. Intra-assay imprecision at 5.79 and 36pmol/l was 2.5 and 3.7% respectively and inter-assay imprecision was 5.7 and 5.1% at 6.18pmol/l and 37.3pmol/l respectively. From 1994, a competitive enzyme immunoassay using a Bayer Immuno 1 System was employed. The intra-assay imprecision was 8.9% at 4.63pmol/l, 3.7% at 16.09pmol/l and 2.7% at 55.98pmol/l.

Since little difference was noted between the two groups throughout puberty for FT4 and a general consensus that there is reasonable consistency among laboratories in this measurement (Dr Tim James and Dr Les Perry - personal communication), it is highly likely that the observations reported are reasonably reliable.

8.10 Comparison of two observers in the ascertainment of PHV age and PHV by the graphical method

	Observer 1			Observer 2			Observer 1-2	
id	PHVage	PHV	PHVSDS	PHVage	PHV	PHVSDS	PHVage	PHV
24	14.95	8.20	-0.49	15.25	6.80	-1.54	-0.30	1.40
25	15.20	9.00	0.33	15.25	8.00	-0.51	-0.05	1.00
28	15.95	8.30	0.33	15.95	8.40	0.33	0.00	-0.10
29	13.80	10.30	0.49	13.75	9.40	-0.26	0.05	0.90
30	12.45	11.30	0.51	12.25	10.80	0.00	0.20	0.50
31	14.25	7.50	-1.11	14.25	8.00	-1.11	0.00	-0.50
33	15.80	9.00	0.77	15.80	9.00	0.77	0.00	0.00
35	12.85	12.20	1.45	12.75	12.20	1.45	0.10	0.00
36	12.25	10.50	0.00	12.25	10.80	0.00	0.00	-0.30
37	12.00	10.90	-0.13	12.00	10.90	-0.13	0.00	0.00
38	15.80	8.20	0.17	15.75	8.40	0.17	0.05	-0.20
39	13.70	7.20	-2.21	13.50	7.20	-2.31	0.20	0.00
40	13.45	8.20	-1.79	13.25	8.00	-1.79	0.20	0.20
41	14.45	11.80	2.05	14.50	11.60	2.05	-0.05	0.20
42	12.50	11.00	0.25	12.50	11.00	0.25	0.00	0.00
43	13.50	10.50	0.73	13.25	11.00	0.73	0.25	-0.50
44	15.25	9.90	1.11	15.25	9.90	1.11	0.00	0.00
45	14.25	10.30	0.85	14.25	10.30	0.85	0.00	0.00
47	15.00	8.60	0.09	15.00	8.80	0.09	0.00	-0.20
46	14.20	10.20	0.66	14.20	10.20	0.66	0.00	0.00
mean	**14.08**	**9.66**	**0.20**	**14.05**	**9.54**	**0.04**	**0.03**	**0.12**
sd	**1.24**	**1.46**	**1.01**	**1.29**	**1.54**	**1.09**	**0.12**	**0.49**

The closeness of the ascertainment of age at PHV and magnitude by this investigator and a colleague working independently was extraordinary and quite unexpected.

8.11 Comparison of PB smoothed curves and hand smoothed for PHV age

PHV Age: Preece Baines (Mario) vs Hand Smoothed (Lynn)

Paired Samples Statistics

STUDY	SEX	PHV age	Mean	sd	sem	N
TIDM	Girls	L	11.92	0.69	0.15	22
		M	12.21	0.97	0.21	22
	Boys	L	14.28	1.23	0.26	23
		M	14.43	1.48	0.31	23
Controls	Girls	L	12.24	0.67	0.11	37
		M	12.44	1.06	0.17	37
	Boys	L	13.74	0.92	0.19	23
		M	13.96	0.95	0.20	23

Paired Samples Test — Paired Differences

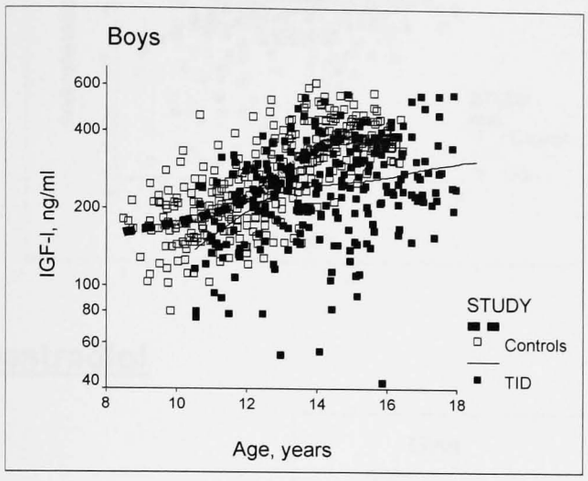
STUDY	SEX	PHVage difference	Mean	sd	sem	95% CI of the Difference Lower	Upper	t	df	Sig.
TIDM	Girls	L-M	-0.29	0.65	0.14	-0.58	0.00	-2.09	21	0.049
	Boys	L-M	-0.15	0.46	0.10	-0.35	0.04	-1.60	22	0.123
Controls	Girls	L-M	-0.20	0.56	0.09	-0.39	-0.01	-2.15	36	0.038
	Boys	L-M	-0.22	0.30	0.06	-0.35	-0.09	-3.59	22	0.002

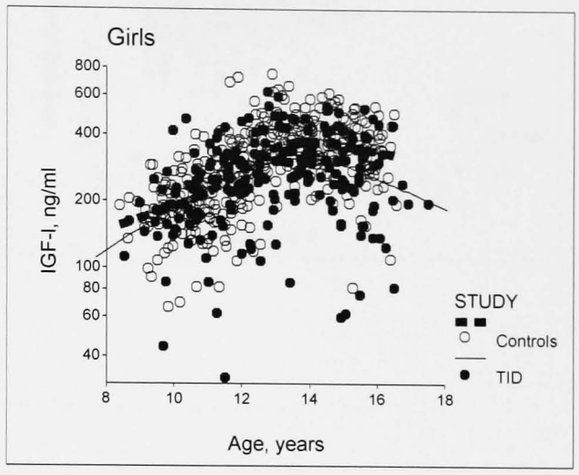
There was a consistent difference with the PB model giving an older PHV age than the hand smoothed one.

8.12 Graphs

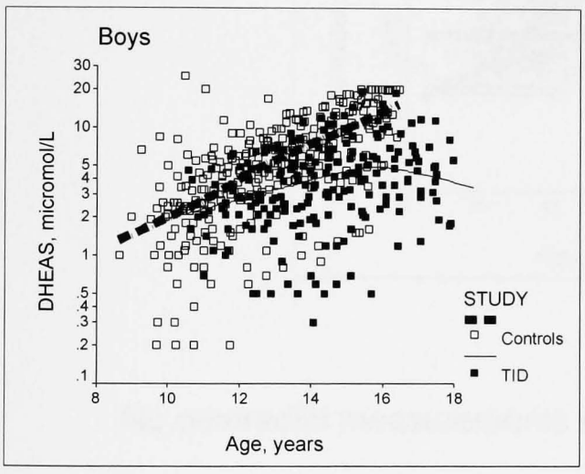
8.12.1 Hormones by Age: comparison of the two cohorts

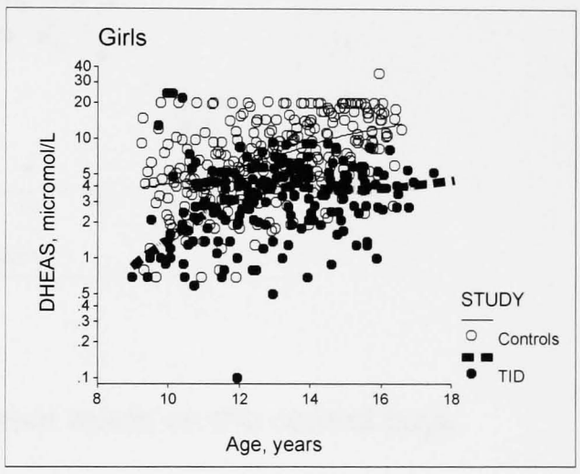
IGF-I



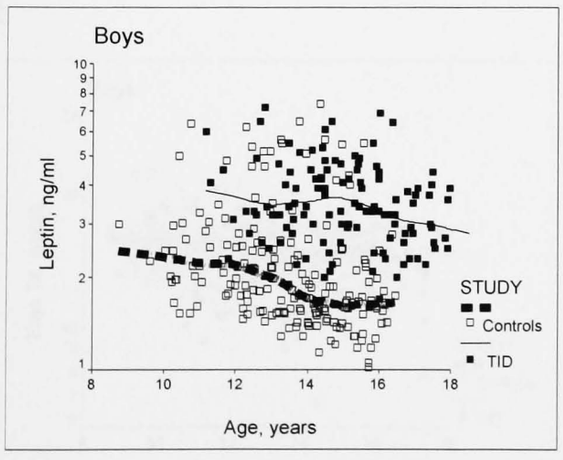


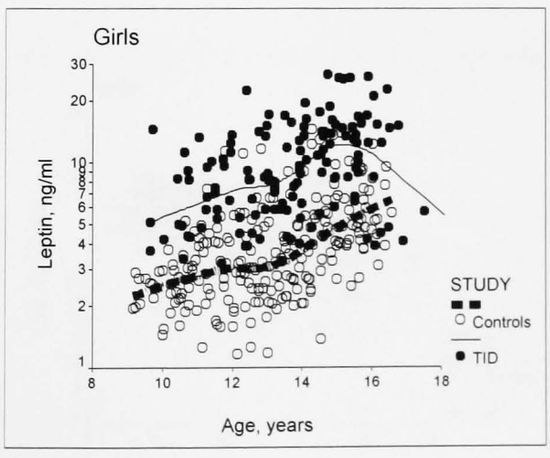
DHEAS



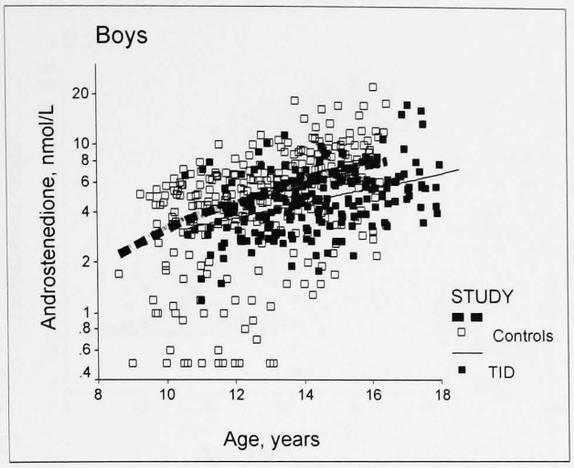


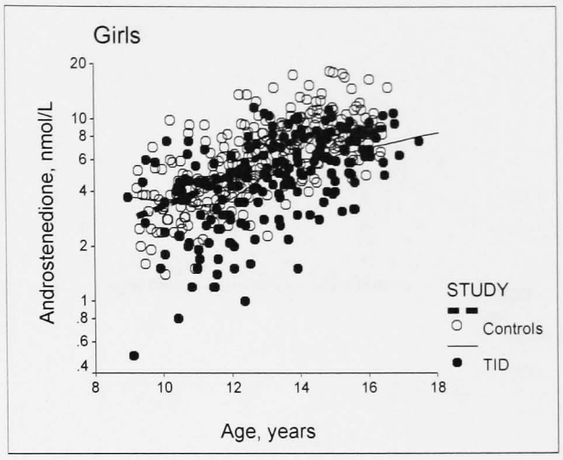
Leptin



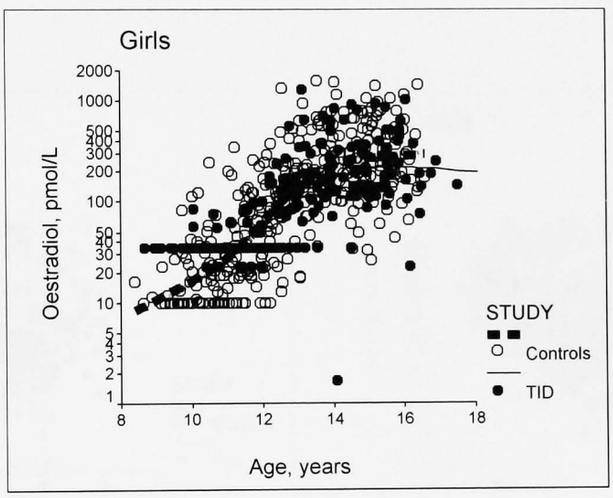


A4



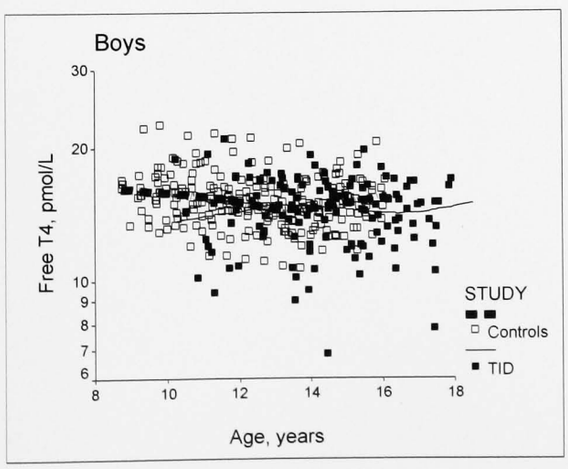


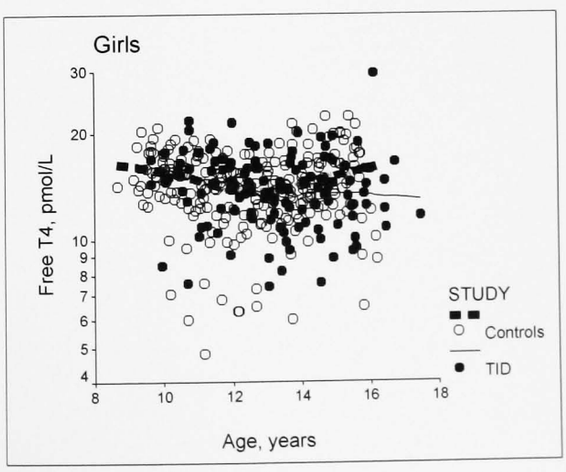
Oestradiol



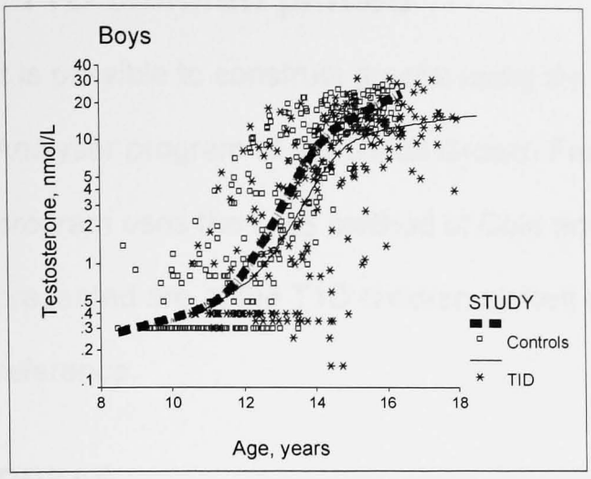
No oestradiol measurements were made on the control boys.

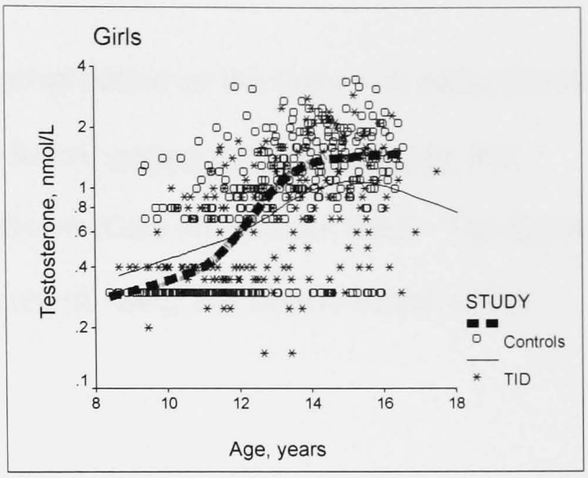
FT4



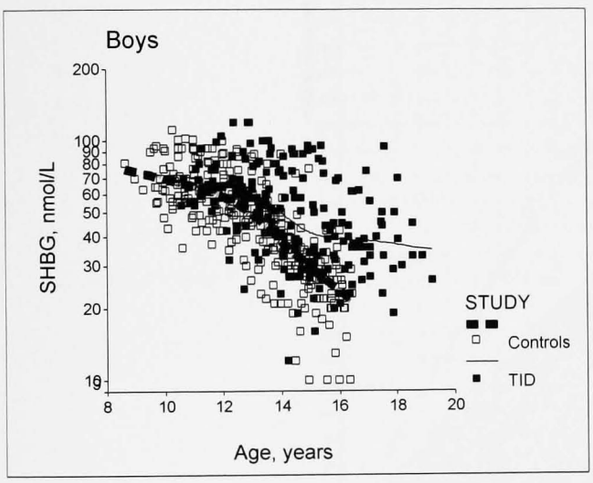


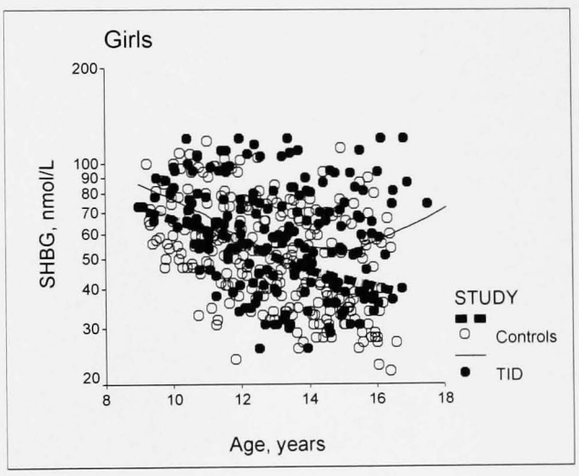
Testosterone





SHBG





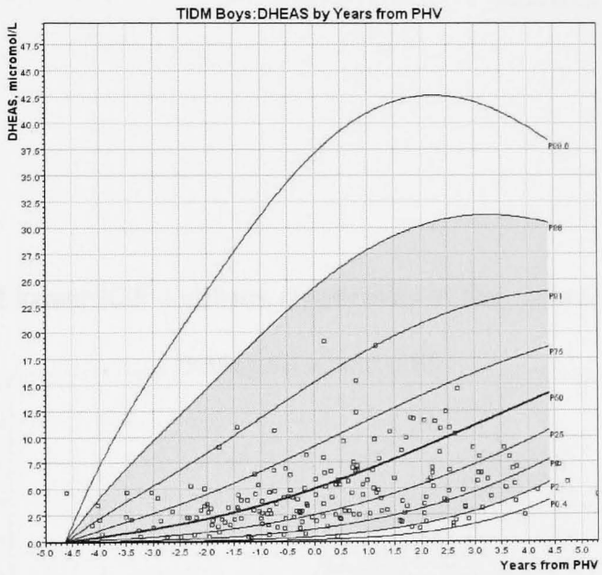
8.12.2 Reference Charts for Hormones by Years from PHV with T1D children plotted

It is possible to construct graphs using the control cohort as the reference data (Growth Analyser program of the Dutch Growth Foundation, www.growthanalyser.org, this program uses the LMS method of Cole and Green (Cole and Green 1992). The figures presented are of the T1D children plotted on charts using this control cohort as the reference.

DHEAS

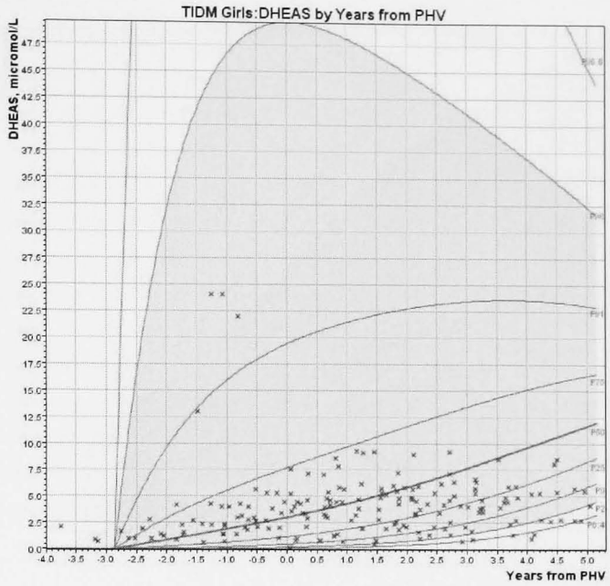
Boys

This demonstrates the lower levels of DHEAS in the T1D boys relative to the controls.



Girls

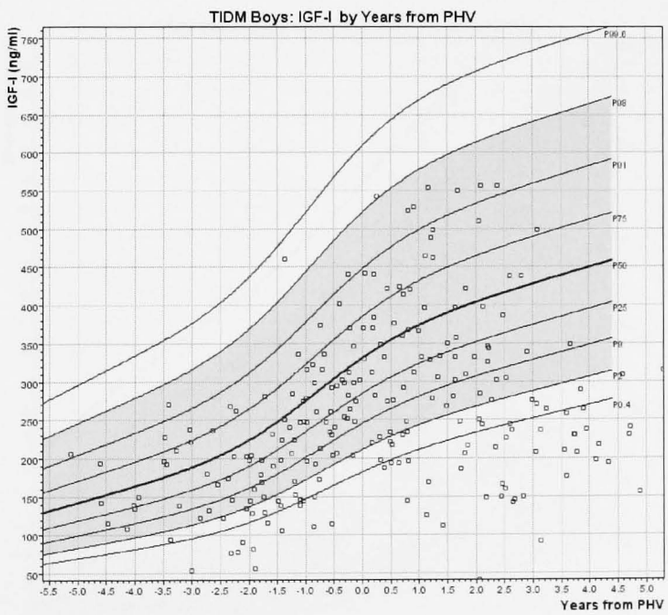
Lower DHEAS levels in the T1D girls are evident relative to the controls:



IGF-I

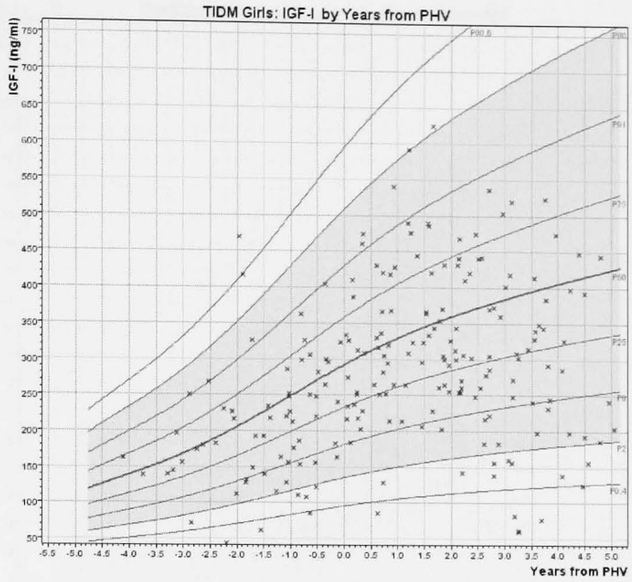
Boys

The preponderance of lower IGF-I values especially in the years after PHV is evident.



Girls

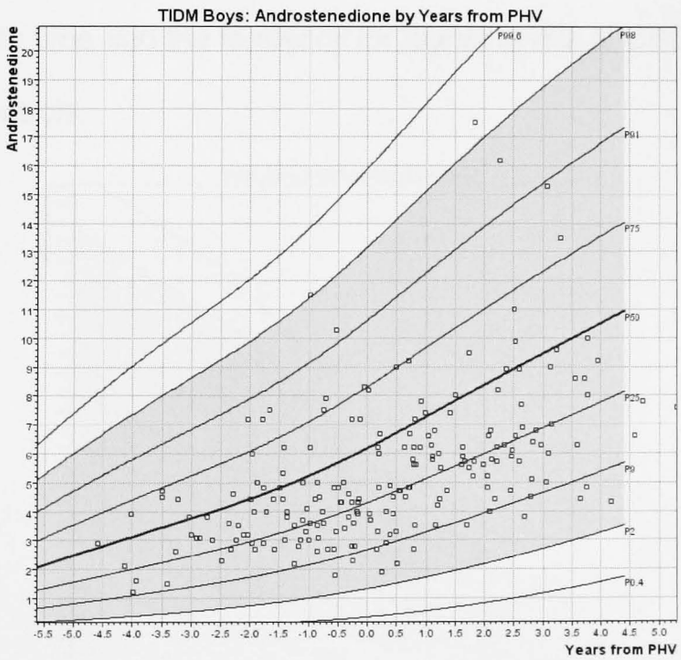
The tendency for lower IGF-I levels in the T1D girls is observed.



A4

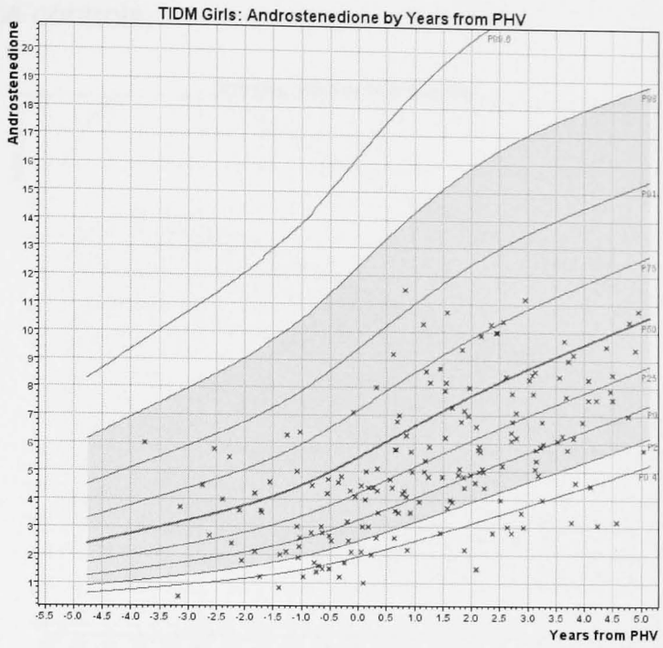
Boys

The trend for lower A4 levels in the T1D boys is seen.



Girls

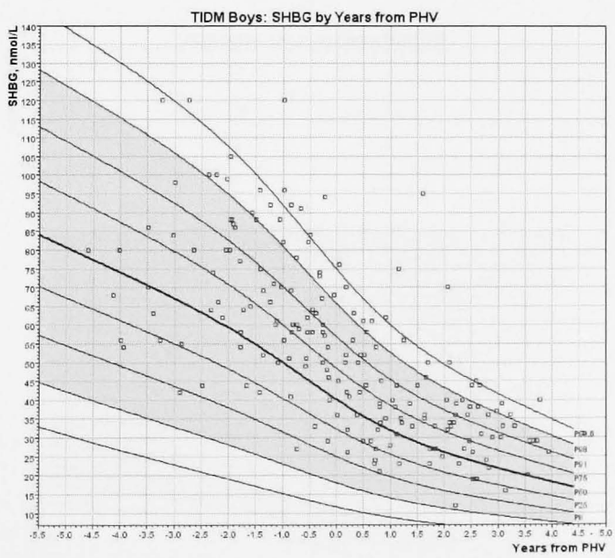
The lower A4 levels of the T1D girls are observed.



SHBG

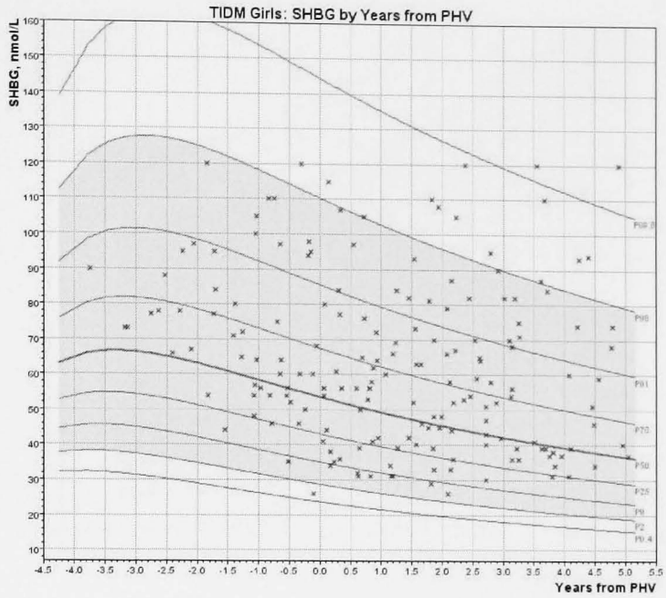
Boys

Note the decline with time and the tendency for higher levels of SHBG in the T1D boys compared to the controls.



Girls

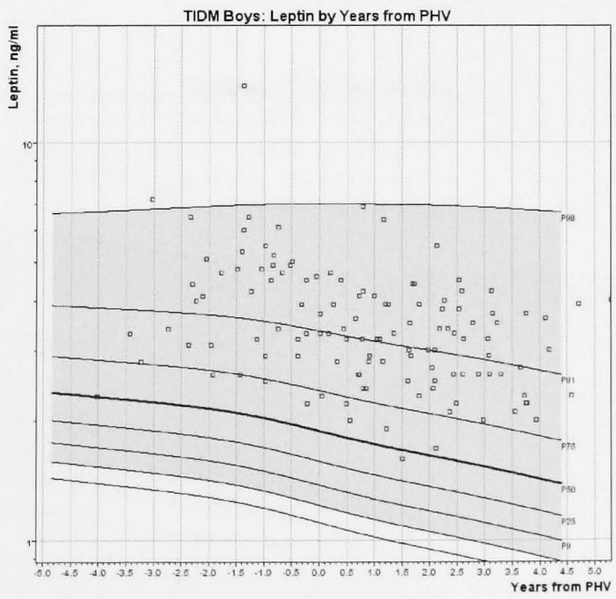
Note the decline with time and a slight tendency for higher levels of SHBG in the T1D girls compared to the controls.



Leptin

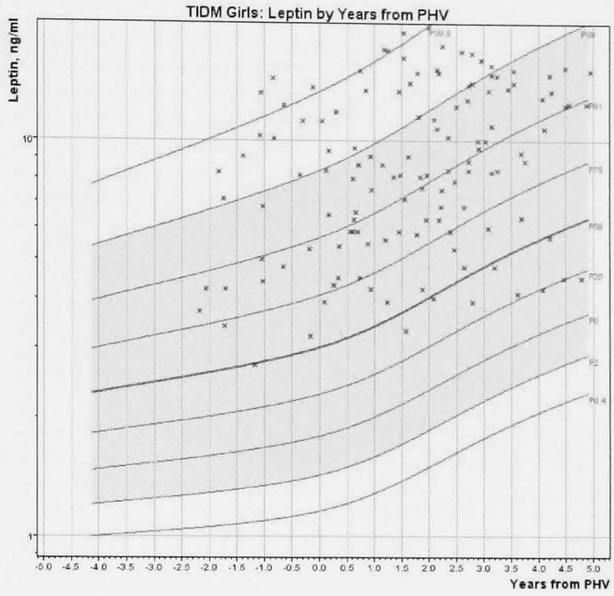
Boys

Note the decline with time and the obviously higher levels of leptin in the T1D boys compared to the controls.



Girls

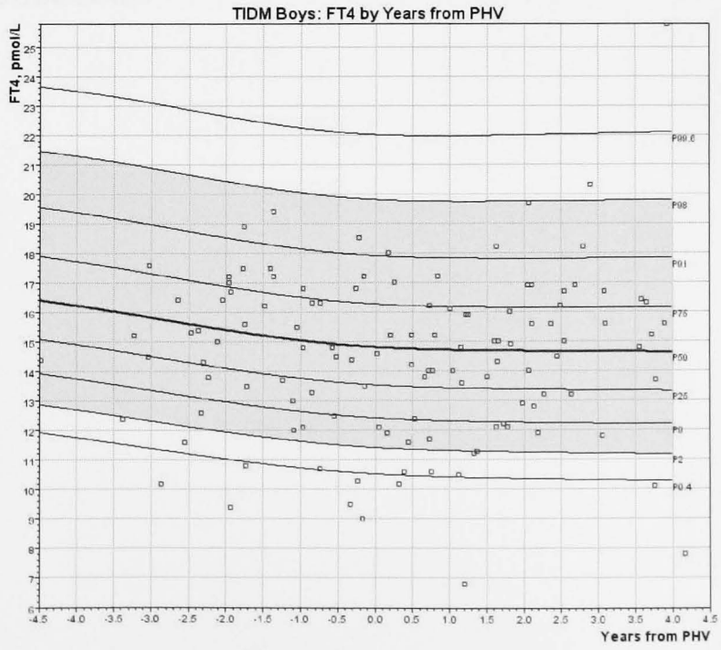
Leptin levels increase with time and there are obviously higher levels of leptin in the T1D girls compared to the controls.



Free Thyroxine

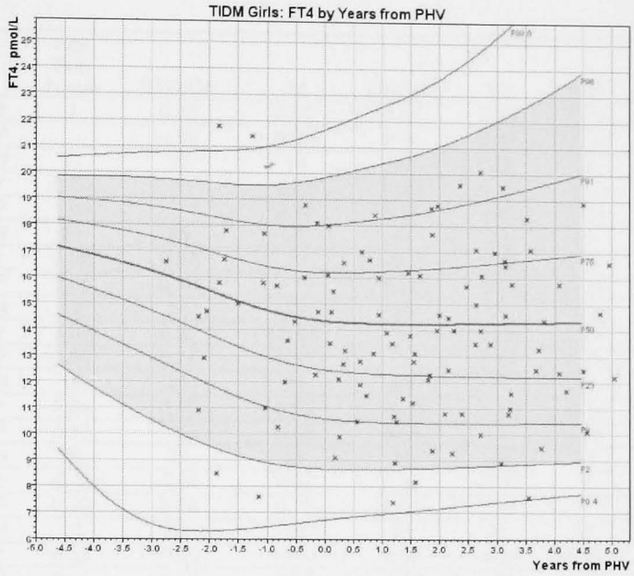
Boys

The reference graph of FT4 by years from PHV based on control data is presented with FT4 levels of the T1D boys plotted on it and not much difference between the two cohorts is noted.



Girls

The reference graph of FT4 by years from PHV based on control data is presented with FT4 levels of the T1D girls plotted on it.



It has not been possible for this investigator to construct the reference charts for testosterone or oestradiol.

8.13 Correlation Matrices

8.13.1 Pubertal Onset

Correlation Matrix for Age at Pubertal Onset and Hormones at the start of Puberty: Girls

			Age Pub Onset	BMISDS	A4	DHEAS	E2	FAI	IGF-I	Leptin	SHBG	Testos
T1D Girls	Age Pub Onset	r	1	0.00	0.05	0.05	0.04	-0.13	0.33	0.22	0.05	-0.08
		P		0.99	0.85	0.85	0.87	0.59	0.14	0.61	0.83	0.75
		N	21	21	19	19	20	19	21	8	19	20
	BMISDS	r	0.00	1	0.37	0.22	0.10	-0.05	-0.02	0.80	0.09	-0.17
		P	0.99		0.10	0.34	0.65	0.84	0.92	0.01	0.69	0.45
		N	21	23	21	21	22	21	23	9	21	22
	A4	r	0.05	0.37	1	-0.01	0.03	0.07	-0.04	0.29	0.07	0.12
		P	0.85	0.10		0.97	0.89	0.76	0.85	0.45	0.77	0.59
		N	19	21	21	21	21	21	21	9	21	21
	DHEAS	r	0.05	0.22	-0.01	1	-0.33	0.34	0.17	0.28	-0.15	0.37
		P	0.85	0.34	0.97		0.14	0.13	0.45	0.47	0.52	0.10
		N	19	21	21	21	21	21	21	9	21	21
	E2	r	0.04	0.10	0.03	-0.33	1	-0.10	-0.05	-	-0.22	-0.26
		P	0.87	0.65	0.89	0.14		0.66	0.82	0.96	0.34	0.24
		N	20	22	21	21	22	21	22	9	21	22
	FAI	r	-0.13	-0.05	0.07	0.34	-0.10	1	0.18	0.79	-0.77	0.74
		P	0.59	0.84	0.76	0.13	0.66		0.43	0.01	0.00	0.00
		N	19	21	21	21	21	21	21	9	21	21
	IGF-I	r	0.33	-0.02	-0.04	0.17	-0.05	0.18	1	0.10	-0.07	0.15
		P	0.14	0.92	0.85	0.45	0.82	0.43		0.79	0.75	0.51
		N	21	23	21	21	22	21	23	9	21	22
	Leptin	r	0.22	0.80	0.29	0.28	-0.02	0.79	0.10	1	-0.45	0.74
		P	0.61	0.01	0.45	0.47	0.96	0.01	0.79		0.22	0.02
		N	8	9	9	9	9	9	9	9	9	9
	SHBG	r	0.05	0.09	0.07	-0.15	-0.22	-0.77	-0.07	-	1	-0.30
		P	0.83	0.69	0.77	0.52	0.34	0.00	0.75	0.22		0.19
		N	19	21	21	21	21	21	21	9	21	21
	Testos	r	-0.08	-0.17	0.12	0.37	-0.26	0.74	0.15	0.74	-0.30	1
		P	0.75	0.45	0.59	0.10	0.24	0.00	0.51	0.02	0.19	
		N	20	22	21	21	22	21	22	9	21	22
Control Girls	AgePub Onset	r	1	-0.65	0.29	-0.27	0.48	0.17	0.11	-	0.03	0.30
		P		0.00	0.27	0.31	0.02	0.53	0.63	0.4	0.92	0.17
		N	31	31	16	16	22	16	22	13	16	22
	BMISDS	r	-0.65	1	-0.18	0.33	-0.18	0.06	-0.05	0.38	-0.33	-0.13
		P	0.00		0.49	0.20	0.40	0.83	0.80	0.20	0.20	0.56
		N	31	52	17	17	23	17	23	13	17	23
	A4	r	0.29	-0.18	1	0.64	0.47	0.60	0.51	-	-0.06	0.66
		P	0.27	0.49		0.01	0.06	0.01	0.04	0.73	0.81	0.00
		N	16	17	17	17	17	17	17	10	17	17
	DHEAS	r	-0.27	0.33	0.64	1	0.21	0.43	0.39	-	-0.30	0.40
		P	0.31	0.20	0.01		0.43	0.09	0.13	0.96	0.24	0.11
		N	16	17	17	17	17	17	17	10	17	17
	E2	r	0.48	-0.18	0.47	0.21	1	0.23	0.40	-	0.09	0.44
		P	0.02	0.40	0.06	0.43		0.37	0.06	0.83	0.74	0.04
		N	22	23	17	17	23	17	23	13	17	23
	FAI	r	0.17	0.06	0.60	0.43	0.23	1	0.57	0.17	-0.50	0.95
		P	0.53	0.83	0.01	0.09	0.37		0.02	0.63	0.04	0.00
		N	16	17	17	17	17	17	17	10	17	17
	IGF-I	r	0.11	-0.05	0.51	0.39	0.40	0.57	1	0.57	-0.40	0.48
		P	0.63	0.80	0.04	0.13	0.06	0.02		0.04	0.11	0.02
		N	22	23	17	17	23	17	23	13	17	23
	Leptin	r	-0.25	0.38	-0.12	-0.02	-0.07	0.17	0.57	1	-0.09	0.08
		P	0.41	0.20	0.73	0.96	0.83	0.63	0.04		0.80	0.78
		N	13	13	10	10	13	10	13	13	10	13
	SHBG	r	0.03	-0.33	-0.06	-0.30	0.09	-0.50	-0.40	-	1	-0.20
		P	0.92	0.20	0.81	0.24	0.74	0.04	0.11	0.80		0.43
		N	16	17	17	17	17	17	17	10	17	17
	Testos	r	0.30	-0.13	0.66	0.40	0.44	0.95	0.48	0.08	-0.20	1
		P	0.17	0.56	0.00	0.11	0.04	0.00	0.02	0.78	0.43	
		N	22	23	17	17	23	17	23	13	17	23

Correlation Matrix for Age at Pubertal Onset and Hormones at the start of Puberty: Boys

			Age Pub Onset	BMISDS	A4	DHEAS	E2	FAI	IGF-I	Leptin	SHBG	Testos
T1D Boys	Age Pub Onset	r	1	-0.38	-0.07	-0.22	0.05	-0.14	0.07	-0.17	0.31	-0.05
		P		0.08	0.76	0.36	0.84	0.57	0.77	0.62	0.20	0.83
		N	23	23	19	19	19	19	21	11	19	21
	BMISDS	r	-0.38	1	-0.19	0.05	0.35	0.00	0.17	0.48	-0.55	0.07
		P	0.08		0.41	0.82	0.11	0.99	0.42	0.14	0.01	0.74
		N	23	26	21	21	22	21	24	11	21	24
	A4	r	-0.07	-0.19	1	0.41	-0.02	0.19	-0.04	-0.18	-0.15	0.15
		P	0.76	0.41		0.07	0.92	0.40	0.87	0.60	0.51	0.51
		N	19	21	21	21	20	21	21	11	21	21
	DHEAS	r	-0.22	0.05	0.41	1	0.11	0.18	-0.05	-0.11	-0.08	0.14
		P	0.36	0.82	0.07		0.64	0.45	0.82	0.74	0.73	0.55
		N	19	21	21	21	20	21	21	11	21	21
	E2	r	0.05	0.35	-0.02	0.11	1	-0.10	-0.14	0.19	0.09	-0.07
		P	0.84	0.11	0.92	0.64		0.68	0.52	0.57	0.71	0.76
		N	19	22	20	20	22	20	22	11	20	22
	FAI	r	-0.14	0.00	0.19	0.18	-0.10	1	0.37	0.56	-0.47	0.98
		P	0.57	0.99	0.40	0.45	0.68		0.10	0.07	0.03	0.00
		N	19	21	21	21	20	21	21	11	21	21
	IGF-I	r	0.07	0.17	-0.04	-0.05	-0.14	0.37	1.00	0.37	-0.18	0.33
		P	0.77	0.42	0.87	0.82	0.52	0.10		0.27	0.43	0.11
		N	21	24	21	21	22	21	24	11	21	24
	Leptin	r	-0.17	0.48	-0.18	-0.11	0.19	0.56	0.37	1.00	-0.61	0.41
		P	0.62	0.14	0.60	0.74	0.57	0.07	0.27		0.04	0.21
		N	11	11	11	11	11	11	11	11	11	11
	SHBG	r	0.31	-0.55	-0.15	-0.08	0.09	-0.47	-0.18	-0.61	1.00	-0.30
		P	0.20	0.01	0.51	0.73	0.71	0.03	0.43	0.04		0.18
		N	19	21	21	21	20	21	21	11	21	21
	Testos	r	-0.05	0.07	0.15	0.14	-0.07	0.98	0.33	0.41	-0.30	1
		P	0.83	0.74	0.51	0.55	0.76	0.00	0.11	0.21	0.18	
		N	21	24	21	21	22	21	24	11	21	24
Control Boys	Age Pub Onset	r	1	0.13	-0.18	0.47		0.25	-0.07	0.30	-0.20	0.24
		P		0.55	0.43	0.03		0.27	0.76	0.23	0.38	0.28
		N	23	23	21	21		21	22	18	21	22
	BMISDS	r	0.13	1	0.07	0.25		0.80	0.42	0.73	-0.58	0.42
		P	0.55		0.74	0.26		0.00	0.04	0.00	0.00	0.05
		N	23	43	22	22		22	23	18	22	23
	A4	r	-0.18	0.07	1	0.06		0.27	-0.11	0.27	-0.13	0.33
		P	0.43	0.74		0.79		0.22	0.63	0.30	0.57	0.13
		N	21	22	22	22		22	22	17	22	22
	DHEAS	r	0.47	0.25	0.06	1		0.56	0.06	0.45	-0.25	0.55
		P	0.03	0.26	0.79			0.01	0.81	0.07	0.26	0.01
		N	21	22	22	22		22	22	17	22	22
	FAI	r	0.25	0.80	0.27	0.56		1	0.48	0.87	-0.61	0.89
	E2					Not available in Control Boys						
		P	0.27	0.00	0.22	0.01			0.02	0.00	0.00	0.00
		N	21	22	22	22		22	22	17	22	22
	IGF-I	r	-0.07	0.42	-0.11	0.06		0.48	1	0.41	-0.24	0.43
		P	0.76	0.04	0.63	0.81		0.02		0.09	0.28	0.04
		N	22	23	22	22		22	23	18	22	23
	Leptin	r	0.30	0.73	0.27	0.45		0.87	0.41	1	-0.56	0.76
		P	0.23	0.00	0.30	0.07		0.00	0.09		0.02	0.00
		N	18	18	17	17		17	18	18	17	18
	SHBG	r	-0.20	-0.58	-0.13	-0.25		-0.61	-0.24	-0.56	1	-0.21
		P	0.38	0.00	0.57	0.26		0.00	0.28	0.02		0.34
		N	21	22	22	22		22	22	17	22	22
	Testos	r	0.24	0.42	0.33	0.55		0.89	0.43	0.76	-0.21	1
		P	0.28	0.05	0.13	0.01		0.00	0.04	0.00	0.34	
		N	22	23	22	22		22	23	18	22	23

Correlation Matrix for Age at Pubertal Onset and Hormones at the start of Puberty: All Boys

		Age Pub Onset	BMISDS	A4	DHEAS	E2	FAI	IGF-I	Leptin	SHBG	Testos
Age Pub Onset	r	1	0.13	-0.10	0.14		-0.01	-0.04	0.40	0.09	0.05
	P		0.40	0.53	0.38		0.95	0.80	0.03	0.58	0.73
	N	46	46	40	40		40	43	29	40	43
BMISDS	r	0.13	1	0.06	0.14		0.33	0.19	0.75	-0.41	0.20
	P	0.40		0.72	0.36		0.03	0.20	0.00	0.01	0.17
	N	46	69	43	43		43	47	29	43	47
A4	r	-0.10	0.06	1	0.14		0.20	-0.08	0.06	-0.11	0.22
	P	0.53	0.72		0.36		0.19	0.61	0.75	0.50	0.15
	N	40	43	43	43		43	43	28	43	43
DHEAS	r	0.14	0.14	0.14	1		0.36	0.00	0.12	-0.20	0.33
	P	0.38	0.36	0.36			0.02	0.98	0.54	0.21	0.03
	N	40	43	43	43		43	43	28	43	43
E2					Not available for Control Boys						
FAI	r	-0.01	0.33	0.20	0.36		1	0.42	0.29	-0.53	0.94
	P	0.95	0.03	0.19	0.02			0.01	0.14	0.00	0.00
	N	40	43	43	43		43	43	28	43	43
IGF-I	r	-0.04	0.19	-0.08	0.00		0.42	1	0.19	-0.22	0.36
	P	0.80	0.20	0.61	0.98		0.01		0.32	0.16	0.01
	N	43	47	43	43		43	47	29	43	47
Leptin	r	0.40	0.75	0.06	0.12		0.29	0.19	1	-0.31	0.20
	P	0.03	0.00	0.75	0.54		0.14	0.32		0.11	0.30
	N	29	29	28	28		28	29	29	28	29
SHBG	r	0.09	-0.41	-0.11	-0.20		-0.53	-0.22	-0.31	1	-0.24
	P	0.58	0.01	0.50	0.21		0.00	0.16	0.11		0.11
	N	40	43	43	43		43	43	28	43	43
Testos	r	0.05	0.20	0.22	0.33		0.94	0.36	0.20	-0.24	1
	P	0.73	0.17	0.15	0.03		0.00	0.01	0.30	0.11	
	N	43	47	43	43		43	47	29	43	47

Correlation Matrix for Age at Pubertal Onset and Hormones at the start of Puberty: All Girls

		Age Pub Onset	BMISDS	A4	DHEAS	E2	FAI	IGF-I	Leptin	SHBG	Testos
Age Pub Onset	r	1	-0.39	0.20	-0.08	0.41	0.11	0.23	-0.04	0.00	0.22
	P		0.00	0.24	0.63	0.01	0.51	0.13	0.86	0.99	0.17
	N	64	64	36	36	43	36	44	21	36	43
BMISDS	r	-0.39	1	-0.04	0.10	-0.12	-0.02	-0.04	0.55	-0.03	-0.12
	P	0.00		0.81	0.55	0.45	0.92	0.77	0.01	0.84	0.44
	N	64	75	38	38	45	38	46	22	38	45
A4	r	0.20	-0.04	1	0.33	0.26	0.34	0.15	-0.21	-0.02	0.37
	P	0.24	0.81		0.04	0.12	0.03	0.37	0.39	0.90	0.02
	N	36	38	38	38	38	38	38	19	38	38
DHEAS	r	-0.08	0.10	0.33	1	0.02	0.38	0.22	-0.44	-0.23	0.34
	P	0.63	0.55	0.04		0.91	0.02	0.18	0.06	0.16	0.04
	N	36	38	38	38	38	38	38	19	38	38
E2	r	0.41	-0.12	0.26	0.02	1	0.15	0.26	-0.12	-0.04	0.31
	P	0.01	0.45	0.12	0.91		0.36	0.08	0.61	0.83	0.04
	N	43	45	38	38	45	38	45	22	38	45
FAI	r	0.11	-0.02	0.34	0.38	0.15	1	0.39	-0.12	-0.59	0.88
	P	0.51	0.92	0.03	0.02	0.36		0.02	0.62	0.00	0.00
	N	36	38	38	38	38	38	38	19	38	38
IGF-I	r	0.23	-0.04	0.15	0.22	0.26	0.39	1	0.27	-0.18	0.35
	P	0.13	0.77	0.37	0.18	0.08	0.02		0.23	0.28	0.02
	N	44	46	38	38	45	38	46	22	38	45
Leptin	r	-0.04	0.55	-0.21	-0.44	-0.12	-0.12	0.27	1	0.18	-0.08
	P	0.86	0.01	0.39	0.06	0.61	0.62	0.23		0.45	0.72
	N	21	22	19	19	22	19	22	22	19	22
SHBG	r	0.00	-0.03	-0.02	-0.23	-0.04	-0.59	-0.18	0.18	1	-0.21
	P	0.99	0.84	0.90	0.16	0.83	0.00	0.28	0.45		0.19
	N	36	38	38	38	38	38	38	19	38	38
Testos	r	0.22	-0.12	0.37	0.34	0.31	0.88	0.35	-0.08	-0.21	1
	P	0.17	0.44	0.02	0.04	0.04	0.00	0.02	0.72	0.19	
	N	43	45	38	38	45	38	45	22	38	45

8.13.2

Correlation Matrix for Duration (Stage 2 to PHV) and Hormones at Stage 2: All Boys

		Dur G2_PHV	BMISDS_G2	A4	DHEAS	E2	FAI	Leptin	SHBG	Testos	IGF-I
Dur G2_PHV	r	1	-0.33	-0.05	-0.23		-0.45	-0.41	0.12	-0.48	-0.24
	P		0.02	0.77	0.15		0.00	0.03	0.46	0.00	0.12
	N	46	46	40	40		40	29	40	43	43
BMISDS_G2	r	-0.33	1	0.06	0.14		0.33	0.75	-0.41	0.20	0.19
	P	0.02		0.72	0.36		0.03	0.00	0.01	0.17	0.20
	N	46	69	43	43		43	29	43	47	47
A4	r	-0.05	0.06	1	0.14		0.20	0.06	-0.11	0.22	-0.08
	P	0.77	0.72		0.36		0.19	0.75	0.50	0.15	0.61
	N	40	43	43	43		43	28	43	43	43
DHEAS	r	-0.23	0.14	0.14	1		0.36	0.12	-0.20	0.33	0.00
	P	0.15	0.36	0.36			0.02	0.54	0.21	0.03	0.98
	N	40	43	43	43		43	28	43	43	43
E2	r				Not available for Control Boys						
FAI	r	-0.45	0.33	0.20	0.36		1	0.29	-0.53	0.94	0.42
	P	0.00	0.03	0.19	0.02			0.14	0.00	0.00	0.01
	N	40	43	43	43		43	28	43	43	43
Leptin	r	-0.41	0.75	0.06	0.12		0.29	1	-0.31	0.20	0.19
	P	0.03	0.00	0.75	0.54		0.14		0.11	0.30	0.32
	N	29	29	28	28		28	29	28	29	29
SHBG	r	0.12	-0.41	-0.11	-0.20		-0.53	-0.31	1	-0.24	-0.22
	P	0.46	0.01	0.50	0.21		0.00	0.11		0.11	0.16
	N	40	43	43	43		43	28	43	43	43
Testos	r	-0.48	0.20	0.22	0.33		0.94	0.20	-0.24	1	0.36
	P	0.00	0.17	0.15	0.03		0.00	0.30	0.11		0.01
	N	43	47	43	43		43	29	43	47	47
IGF-I	r	-0.24	0.19	-0.08	0.00		0.42	0.19	-0.22	0.36	1
	P	0.12	0.20	0.61	0.98		0.01	0.32	0.16	0.01	
	N	43	47	43	43		43	29	43	47	47

Correlation Matrix for Duration (Stage 2 to PHV) and Hormones at Stage 2: All Girls

All Girls		BMISDS_B2	A4	DHEAS	E2	FAI	IGF-I	Leptin	SHBG	Testos	Dur
BMISDS_B2	r	1	-0.04	0.10	-0.12	-0.02	-0.04	0.55	-0.03	-0.12	0.32
	P		0.81	0.55	0.45	0.92	0.77	0.01	0.84	0.44	0.02
	N	75	38	38	45	38	46	22	38	45	53
A4	r	-0.04	1	0.33	0.26	0.34	0.15	-0.21	-0.02	0.37	-0.08
	P	0.81		0.04	0.12	0.03	0.37	0.39	0.90	0.02	0.63
	N	38	38	38	38	38	38	19	38	38	36
DHEAS	r	0.10	0.33	1	0.02	0.38	0.22	-0.44	-0.23	0.34	0.17
	P	0.55	0.04		0.91	0.02	0.18	0.06	0.16	0.04	0.32
	N	38	38	38	38	38	38	19	38	38	36
E2	r	-0.12	0.26	0.02	1	0.15	0.26	-0.12	-0.04	0.31	-0.35
	P	0.45	0.12	0.91		0.36	0.08	0.61	0.83	0.04	0.02
	N	45	38	38	45	38	45	22	38	45	43
FAI	r	-0.02	0.34	0.38	0.15	1	0.39	-0.12	-0.59	0.88	-0.13
	P	0.92	0.03	0.02	0.36		0.02	0.62	0.00	0.00	0.46
	N	38	38	38	38	38	38	19	38	38	36
IGF-I	r	-0.04	0.15	0.22	0.26	0.39	1	0.27	-0.18	0.35	-0.47
	P	0.77	0.37	0.18	0.08	0.02		0.23	0.28	0.02	0.00
	N	46	38	38	45	38	46	22	38	45	44
Leptin	r	0.55	-0.21	-0.44	-0.12	-0.12	0.27	1	0.18	-0.08	-0.06
	P	0.01	0.39	0.06	0.61	0.62	0.23		0.45	0.72	0.80
	N	22	19	19	22	19	22	22	19	22	21
SHBG	r	-0.03	-0.02	-0.23	-0.04	-0.59	-0.18	0.18	1	-0.21	-0.05
	P	0.84	0.90	0.16	0.83	0.00	0.28	0.45		0.19	0.78
	N	38	38	38	38	38	38	19	38	38	36
Testos	r	-0.12	0.37	0.34	0.31	0.88	0.35	-0.08	-0.21	1	-0.25
	P	0.44	0.02	0.04	0.04	0.00	0.02	0.72	0.19		0.10
	N	45	38	38	45	38	45	22	38	45	43
Dur B2-PHV	r	0.32	-0.08	0.17	-0.35	-0.13	-0.47	-0.06	-0.05	-0.25	1
	P	0.02	0.63	0.32	0.02	0.46	0.00	0.80	0.78	0.10	
	N	53	36	36	43	36	44	21	36	43	53

Correlation Matrix for Duration (Stage 2 to PHV) and Hormones at Stage 2: Control Boys

			BMISD S_G2	A4	DHEAS	E2	FAI	Leptin	SHBG	testos	IGF-I	Dur G2_PHV
Control Boys	BMISD S_G2	r	1	0.07	0.25		0.80	0.73	-0.58	0.42	0.42	-0.33
		P		0.74	0.26		0.00	0.00	0.00	0.05	0.04	0.13
		N	43	22	22		22	18	22	23	23	23
	A4	r	0.07	1	0.06		0.27	0.27	-0.13	0.33	-0.11	0.06
		P	0.74		0.79		0.22	0.30	0.57	0.13	0.63	0.78
		N	22	22	22		22	17	22	22	22	21
	DHEAS	r	0.25	0.06	1.00		0.56	0.45	-0.25	0.55	0.06	-0.33
		P	0.26	0.79			0.01	0.07	0.26	0.01	0.81	0.14
		N	22	22	22		22	17	22	22	22	21
	E2	Not Available for Control Boys										
	FAI	r	0.80	0.27	0.56		1.00	0.87	-0.61	0.89	0.48	-0.44
		P	0.00	0.22	0.01			0.00	0.00	0.00	0.02	0.05
		N	22	22	22		22	17	22	22	22	21
	Leptin	r	0.73	0.27	0.45		0.87	1	-0.56	0.76	0.41	-0.67
		P	0.00	0.30	0.07		0.00		0.02	0.00	0.09	0.00
		N	18	17	17		17	18	17	18	18	18
	SHBG	r	-0.58	-0.13	-0.25		-0.61	-0.56	1	-0.21	-0.24	0.19
		P	0.00	0.57	0.26		0.00	0.02		0.34	0.28	0.41
		N	22	22	22		22	17	22	22	22	21
	testos	r	0.42	0.33	0.55		0.89	0.76	-0.21	1	0.43	-0.50
		P	0.05	0.13	0.01		0.00	0.00	0.34		0.04	0.02
		N	23	22	22		22	18	22	23	23	22
	IGF-I	r	0.42	-0.11	0.06		0.48	0.41	-0.24	0.43	1	-0.45
		P	0.04	0.63	0.81		0.02	0.09	0.28	0.04		0.03
		N	23	22	22		22	18	22	23	23	22
	Dur G2_PHV	r	-0.33	0.06	-0.33		-0.44	-0.67	0.19	-0.50	-0.45	1
		P	0.13	0.78	0.14		0.05	0.00	0.41	0.02	0.03	
		N	23	21	21		21	18	21	22	22	23

Correlation Matrix for Duration (Stage 2 to PHV) and Hormones at Stage 2: Control Girls

			BMISD S_B2	A4	DHEAS	E2	FAI	Leptin	SHBG	testos	IGF-I	Dur B2_PHV
Control Girls	BMISD S_B2	r	1	-0.18	0.33	-0.18	0.06	0.38	-0.33	-0.13	-0.05	0.44
		P		0.49	0.20	0.40	0.83	0.20	0.20	0.56	0.80	0.01
		N	52	17	17	23	17	13	17	23	23	32
	A4	r	-0.18	1	0.64	0.47	0.60	-0.12	-0.06	0.66	0.51	-0.17
		P	0.49		0.01	0.06	0.01	0.73	0.81	0.00	0.04	0.51
		N	17	17	17	17	17	10	17	17	17	17
	DHEAS	r	0.33	0.64	1	0.21	0.43	-0.02	-0.30	0.40	0.39	0.24
		P	0.20	0.01		0.43	0.09	0.96	0.24	0.11	0.13	0.36
		N	17	17	17	17	17	10	17	17	17	17
	E2	r	-0.18	0.47	0.21	1	0.23	-0.07	0.09	0.44	0.40	-0.43
		P	0.40	0.06	0.43		0.37	0.83	0.74	0.04	0.06	0.04
		N	23	17	17	23	17	13	17	23	23	23
	FAI	r	0.06	0.60	0.43	0.23	1	0.17	-0.50	0.95	0.57	-0.33
		P	0.83	0.01	0.09	0.37		0.63	0.04	0.00	0.02	0.19
		N	17	17	17	17	17	10	17	17	17	17
	Leptin	r	0.38	-0.12	-0.02	-0.07	0.17	1	-0.09	0.08	0.57	0.34
		P	0.20	0.73	0.96	0.83	0.63		0.80	0.78	0.04	0.26
		N	13	10	10	13	10	13	10	13	13	13
	SHBG	r	-0.33	-0.06	-0.30	0.09	-0.50	-0.09	1	-0.20	-0.40	0.04
		P	0.20	0.81	0.24	0.74	0.04	0.80		0.43	0.11	0.88
		N	17	17	17	17	17	10	17	17	17	17
	testos	r	-0.13	0.66	0.40	0.44	0.95	0.08	-0.20	1	0.48	-0.40
		P	0.56	0.00	0.11	0.04	0.00	0.78	0.43		0.02	0.06
		N	23	17	17	23	17	13	17	23	23	23
	IGF-I	r	-0.05	0.51	0.39	0.40	0.57	0.57	-0.40	0.48	1	-0.45
		P	0.80	0.04	0.13	0.06	0.02	0.04	0.11	0.02		0.03
		N	23	17	17	23	17	13	17	23	23	23
	Dur B2_PHV	r	0.44	-0.17	0.24	-0.43	-0.33	0.34	0.04	-0.40	-0.45	1
		P	0.01	0.51	0.36	0.04	0.19	0.26	0.88	0.06	0.03	
		N	32	17	17	23	17	13	17	23	23	32

Correlation Matrix for Duration (Stage 2 to PHV) and Hormones at Stage 2: T1D Boys

			BMIS DS_G 2	A4	DHEA S	E2	FAI	Lepti n	SHB G	testo	IGF-I	Dur G2_P HV	in_kg	HbA 1c
T1D Boys	BMIS DS_ G2	r	1	-	0.05	0.35	0.00	0.48	-0.55	0.07	0.17	-0.10	0.25	0.00
		P		0.41	0.82	0.11	0.99	0.14	0.01	0.74	0.42	0.65	0.23	1.00
		N	26	21	21	22	21	11	21	24	24	23	24	26
	A4	r	-0.19	1	0.41	-0.02	0.19	-0.18	-0.15	0.15	-0.04	-0.25	-0.38	-0.10
		P	0.41		0.07	0.92	0.40	0.60	0.51	0.51	0.87	0.31	0.11	0.67
		N	21	21	21	20	21	11	21	21	21	19	19	21
	DHE AS	r	0.05	0.41	1	0.11	0.18	-0.11	-0.08	0.14	-0.05	-0.16	-0.28	0.01
		P	0.82	0.07		0.64	0.45	0.74	0.73	0.55	0.82	0.51	0.25	0.97
		N	21	21	21	20	21	11	21	21	21	19	19	21
	E2	r	0.35	-	0.11	1	-	0.19	0.09	-0.07	-0.14	0.20	0.21	-0.13
		P	0.11	0.92	0.64		0.68	0.57	0.71	0.76	0.52	0.41	0.38	0.58
		N	22	20	20	22	20	11	20	22	22	19	20	22
	FAI	r	0.00	0.19	0.18	-0.10	1	0.56	-0.47	0.98	0.37	-0.57	0.45	0.32
		P	0.99	0.40	0.45	0.68		0.07	0.03	0.00	0.10	0.01	0.06	0.16
		N	21	21	21	20	21	11	21	21	21	19	19	21
	Lepti	r	0.48	-	-0.11	0.19	0.56	1	-0.61	0.41	0.37	0.15	0.44	0.04
		P	0.14	0.60	0.74	0.57	0.07		0.04	0.21	0.27	0.65	0.17	0.91
		N	11	11	11	11	11	11	11	11	11	11	11	11
	SHB	r	-0.55	-	-0.08	0.09	-	-0.61	1	-0.30	-0.18	0.22	-0.32	-0.17
		P	0.01	0.51	0.73	0.71	0.03	0.04		0.18	0.43	0.36	0.18	0.47
		N	21	21	21	20	21	11	21	21	21	19	19	21
	testo	r	0.07	0.15	0.14	-0.07	0.98	0.41	-0.30	1	0.33	-0.56	0.29	0.37
		P	0.74	0.51	0.55	0.76	0.00	0.21	0.18		0.11	0.01	0.19	0.08
		N	24	21	21	22	21	11	21	24	24	21	22	24
	IGF-I	r	0.17	-	-0.05	-0.14	0.37	0.37	-0.18	0.33	1	-0.20	-0.11	-0.04
		P	0.42	0.87	0.82	0.52	0.10	0.27	0.43	0.11		0.38	0.64	0.84
		N	24	21	21	22	21	11	21	24	24	21	22	24
	Dur G2_P	r	-0.10	-	-0.16	0.20	-	0.15	0.22	-0.56	-0.20	1	-0.08	-0.44
		P	0.65	0.31	0.51	0.41	0.01	0.65	0.36	0.01	0.38		0.73	0.04
		N	23	19	19	19	19	11	19	21	21	23	23	23
	in_kg	r	0.25	-	-0.28	0.21	0.45	0.44	-0.32	0.29	-0.11	-0.08	1	0.26
		P	0.23	0.11	0.25	0.38	0.06	0.17	0.18	0.19	0.64	0.73		0.23
		N	24	19	19	20	19	11	19	22	22	23	24	24
	HbA1	r	0.00	-	0.01	-0.13	0.32	0.04	-0.17	0.37	-0.04	-0.44	0.26	1
		P	1.00	0.67	0.97	0.58	0.16	0.91	0.47	0.08	0.84	0.04	0.23	
		N	26	21	21	22	21	11	21	24	24	23	24	26

Correlation Matrix for Duration (Stage 2 to PHV) and Hormones at Stage 2: T1D Girls

			BMISDS_B2	A4	DHEAS	E2	FAI	Leptin	SHBG	testos	IGF-I	Dur B2_PHV	in_kg	HbA1c
T1D Girls	BMISDS_B2	r	1	0.37	0.22	0.10	-0.05	0.80	0.09	-0.17	-0.02	0.15	-0.31	0.07
		P		0.10	0.34	0.65	0.84	0.01	0.69	0.45	0.92	0.52	0.17	0.74
		N	23	21	21	22	21	9	21	22	23	21	21	23
	A4	r	0.37	1	-0.01	0.03	0.07	0.29	0.07	0.12	-0.04	-0.11	0.10	-0.11
		P	0.10		0.97	0.89	0.76	0.45	0.77	0.59	0.85	0.67	0.68	0.63
		N	21	21	21	21	21	9	21	21	21	19	19	21
	DHEAS	r	0.22	-0.01	1	-0.33	0.34	0.28	-0.15	0.37	0.17	0.05	-0.14	0.24
		P	0.34	0.97		0.14	0.13	0.47	0.52	0.10	0.45	0.84	0.56	0.30
		N	21	21	21	21	21	9	21	21	21	19	19	21
	E2	r	0.10	0.03	-0.33	1	-0.10	-0.02	-0.22	-0.26	-0.05	-0.10	0.45	0.21
		P	0.65	0.89	0.14		0.66	0.96	0.34	0.24	0.82	0.67	0.05	0.35
		N	22	21	21	22	21	9	21	22	22	20	20	22
	FAI	r	-0.05	0.07	0.34	-0.10	1	0.79	-0.77	0.74	0.18	0.18	-0.24	-0.13
		P	0.84	0.76	0.13	0.66		0.01	0.00	0.00	0.43	0.46	0.32	0.57
		N	21	21	21	21	21	9	21	21	21	19	19	21
	Leptin	r	0.80	0.29	0.28	-0.02	0.79	1	-0.45	0.74	0.10	0.02	-0.46	-0.17
		P	0.01	0.45	0.47	0.96	0.01		0.22	0.02	0.79	0.95	0.25	0.66
		N	9	9	9	9	9	9	9	9	9	8	8	9
	SHBG	r	0.09	0.07	-0.15	-0.22	-0.77	-0.45	1	-0.30	-0.07	-0.09	0.04	-0.16
		P	0.69	0.77	0.52	0.34	0.00	0.22		0.19	0.75	0.73	0.88	0.48
		N	21	21	21	21	21	9	21	21	21	19	19	21
	testos	r	-0.17	0.12	0.37	-0.26	0.74	0.74	-0.30	1	0.15	0.10	0.09	-0.35
		P	0.45	0.59	0.10	0.24	0.00	0.02	0.19		0.51	0.69	0.70	0.11
		N	22	21	21	22	21	9	21	22	22	20	20	22
	IGF-I	r	-0.02	-0.04	0.17	-0.05	0.18	0.10	-0.07	0.15	1	-0.53	0.47	0.36
		P	0.92	0.85	0.45	0.82	0.43	0.79	0.75	0.51		0.01	0.03	0.09
		N	23	21	21	22	21	9	21	22	23	21	21	23
	Dur B2_PHV	r	0.15	-0.11	0.05	-0.10	0.18	0.02	-0.09	0.10	-0.53	1	-0.48	-0.34
		P	0.52	0.67	0.84	0.67	0.46	0.95	0.73	0.69	0.01		0.03	0.13
		N	21	19	19	20	19	8	19	20	21	21	21	21
	in_kg	r	-0.31	0.10	-0.14	0.45	-0.24	-0.46	0.04	0.09	0.47	-0.48	1	0.36
		P	0.17	0.68	0.56	0.05	0.32	0.25	0.88	0.70	0.03	0.03		0.11
		N	21	19	19	20	19	8	19	20	21	21	21	21
	HbA1c	r	0.07	-0.11	0.24	0.21	-0.13	-0.17	-0.16	-0.35	0.36	-0.34	0.36	1
		P	0.74	0.63	0.30	0.35	0.57	0.66	0.48	0.11	0.09	0.13	0.11	
		N	23	21	21	22	21	9	21	22	23	21	21	23

8.13.3 HtSDS change from Stage 2 to PHV

Correlation Matrix for HtSDS change and Hormones averaged from B2-PHV: All Boys

			HtSDSΔ PHV-G2	BMISDS_G2	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI	Age G2	Dur G2_PHV
All Boys	HtSDS Δ PHV-	r	1	-0.39	-0.01	0.07	-0.16		-	0.21	-	-0.25	-0.41	0.78
		P	.	0.01	0.93	0.66	0.31		0.01	0.18	0.1	0.11	0.01	0.00
		N	45	45	44	43	43		34	43	44	43	45	45
	BMISD	r	-0.39	1	0.20	-0.15	0.18		0.74	-0.40	-	0.16	0.13	-
		P	0.01	.	0.18	0.33	0.25		0.00	0.01	0.7	0.29	0.40	0.02
		N	45	46	45	44	44		35	44	45	44	46	46
	IGF-I	r	-0.01	0.20	1	-0.12	0.10		-	-0.20	0.2	0.31	-0.15	-
		P	0.93	0.18	.	0.43	0.51		0.96	0.18	0.0	0.04	0.33	0.15
		N	44	45	46	45	45		36	45	46	45	45	45
	A4	r	0.07	-0.15	-0.12	1	0.16		-	-0.09	0.2	0.23	-0.22	0.09
		P	0.66	0.33	0.43	.	0.29		0.60	0.54	0.2	0.13	0.15	0.56
		N	43	44	45	45	45		35	45	45	45	44	44
	DHEAS	r	-0.16	0.18	0.10	0.16	1		0.03	-0.36	0.2	0.40	-0.11	-
		P	0.31	0.25	0.51	0.29	.		0.85	0.02	0.0	0.01	0.48	0.38
		N	43	44	45	45	45		35	45	45	45	44	44
	E2	Not available for Control Boys												
	Leptin	r	-0.42	0.74	-0.01	-0.09	0.03		1	-0.12	-	-0.13	0.50	-
		P	0.01	0.00	0.96	0.60	0.85		.	0.50	0.2	0.44	0.00	0.08
		N	34	35	36	35	35		36	35	36	35	35	35
	SHBG	r	0.21	-0.40	-0.20	-0.09	-0.36		-	1	-	-0.56	0.09	0.15
		P	0.18	0.01	0.18	0.54	0.02		0.50	.	0.1	0.00	0.58	0.32
		N	43	44	45	45	45		35	45	45	45	44	44
	Testos	r	-0.23	-0.06	0.29	0.20	0.28		-	-0.22	1	0.90	0.09	-
		P	0.14	0.71	0.05	0.20	0.06		0.25	0.15	.	0.00	0.57	0.00
		N	44	45	46	45	45		36	45	46	45	45	45
	FAI	r	-0.25	0.16	0.31	0.23	0.40		-	-0.56	0.9	1	-0.01	-
		P	0.11	0.29	0.04	0.13	0.01		0.44	0.00	0.0	.	0.94	0.00
		N	43	44	45	45	45		35	45	45	45	44	44
	Age G2	r	-0.41	0.13	-0.15	-0.22	-0.11		0.50	0.09	0.0	-0.01	1	-
		P	0.01	0.40	0.33	0.15	0.48		0.00	0.58	0.5	0.94	.	0.07
		N	45	46	45	44	44		35	44	45	44	46	46
	Dur	r	0.78	-0.33	-0.22	0.09	-0.14		-	0.15	-	-0.46	-0.27	1
		P	0.00	0.02	0.15	0.56	0.38		0.08	0.32	0.0	0.00	0.07	.
		N	45	46	45	44	44		35	44	45	44	46	46

Correlation Matrix for HtSDS change and Hormones averaged from B2-PHV: All Girls

			HtSDS Δ PHV-B2	BMI SDS_B2	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI	Age B2	Dur B2_PHV
All Girls	HtSDS Δ PHV-B2	r	1	0.25	-0.08	0.21	0.32	-0.06	0.09	-0.20	-0.03	0.17	-0.66	0.82
		P	.	0.08	0.61	0.24	0.06	0.69	0.64	0.24	0.86	0.32	0.00	0.00
		N	52	52	42	35	35	42	27	35	42	35	52	51
	BMISDS_B2	r	0.25	1	-0.04	-0.11	0.05	-0.10	0.64	-0.12	-0.08	0.04	-0.50	0.32
		P	0.08	.	0.80	0.53	0.78	0.51	0.00	0.51	0.61	0.81	0.00	0.02
		N	52	52	42	35	35	42	27	35	42	35	52	51
	IGF-I	r	-0.08	-0.04	1	-0.05	0.24	0.08	-0.11	-0.10	0.21	0.24	0.11	-0.35
		P	0.61	0.80	.	0.77	0.14	0.60	0.56	0.54	0.16	0.14	0.47	0.02
		N	42	42	48	40	40	47	32	40	48	40	43	42
	A4	r	0.21	-0.11	-0.05	1	0.47	0.30	-0.31	0.03	0.46	0.43	0.03	0.14
		P	0.24	0.53	0.77	.	0.00	0.07	0.11	0.87	0.00	0.01	0.85	0.43
		N	35	35	40	40	40	39	28	40	40	40	36	36
	DHEAS	r	0.32	0.05	0.24	0.47	1	0.23	-0.32	-0.14	0.44	0.46	-0.19	0.22
		P	0.06	0.78	0.14	0.00	.	0.15	0.10	0.38	0.00	0.00	0.28	0.20
		N	35	35	40	40	40	39	28	40	40	40	36	36
	E2	r	-0.06	-0.10	0.08	0.30	0.23	1	-0.21	0.00	0.29	0.22	0.25	-0.11
		P	0.69	0.51	0.60	0.07	0.15	.	0.26	0.99	0.04	0.17	0.11	0.49
		N	42	42	47	39	39	47	31	39	47	39	43	42
	Leptin	r	0.09	0.64	-0.11	-0.31	-0.32	-0.21	1	0.00	-0.19	-0.16	-0.31	0.24
		P	0.64	0.00	0.56	0.11	0.10	0.26	.	0.98	0.30	0.41	0.12	0.23
		N	27	27	32	28	28	31	32	28	32	28	27	27
	SHBG	r	-0.20	-0.12	-0.10	0.03	-0.14	0.00	0.00	1	-0.08	-0.50	0.10	-0.19
		P	0.24	0.51	0.54	0.87	0.38	0.99	0.98	.	0.64	0.00	0.57	0.26
		N	35	35	40	40	40	39	28	40	40	40	36	36
	Testos	r	-0.03	-0.08	0.21	0.46	0.44	0.29	-0.19	-0.08	1	0.86	-0.01	-0.08
		P	0.86	0.61	0.16	0.00	0.00	0.04	0.30	0.64	.	0.00	0.94	0.62
		N	42	42	48	40	40	47	32	40	48	40	43	42
	FAI	r	0.17	0.04	0.24	0.43	0.46	0.22	-0.16	-0.50	0.86	1	-0.11	0.12
		P	0.32	0.81	0.14	0.01	0.00	0.17	0.41	0.00	0.00	.	0.52	0.48
		N	35	35	40	40	40	39	28	40	40	40	36	36
	Age B2	r	-0.66	-0.50	0.11	0.03	-0.19	0.25	-0.31	0.10	-0.01	-0.11	1	-0.58
		P	0.00	0.00	0.47	0.85	0.28	0.11	0.12	0.57	0.94	0.52	.	0.00
		N	52	52	43	36	36	43	27	36	43	36	53	52
	Dur B2_PHV	r	0.82	0.32	-0.35	0.14	0.22	-0.11	0.24	-0.19	-0.08	0.12	-0.58	1
		P	0.00	0.02	0.02	0.43	0.20	0.49	0.23	0.26	0.62	0.48	0.00	.
		N	51	51	42	36	36	42	27	36	42	36	52	52

Correlation Matrix for HtSDS change and Hormones averaged from G2 to PHV: Control Boys

			HtSDS Δ PHV-B2	BMI SDS _G2	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI	Age G2	Dur G2_PHV
Control Boys	HtSDS Δ PHV-G2	r	1	-0.34	-0.09	0.06	-0.38		-0.59	0.27	-0.19	-0.27	-0.53	0.74
		P	.	0.11	0.69	0.80	0.09		0.01	0.23	0.40	0.23	0.01	0.00
		N	23	23	22	21	21		19	21	22	21	23	23
	BMISDS_G2	r	-0.34	1	0.40	-0.13	0.27		0.77	-0.62	0.21	0.63	0.13	-0.33
		P	0.11	.	0.07	0.57	0.23		0.00	0.00	0.36	0.00	0.55	0.13
		N	23	23	22	21	21		19	21	22	21	23	23
	IGF-I	r	-0.09	0.40	1	-0.39	0.01		0.11	-0.31	0.29	0.46	0.05	-0.36
		P	0.69	0.07	.	0.08	0.97		0.64	0.16	0.18	0.03	0.83	0.10
		N	22	22	23	22	22		20	22	23	22	22	22
	A4	r	0.06	-0.13	-0.39	1	0.01		0.28	0.07	0.09	0.02	-0.29	0.24
		P	0.80	0.57	0.08	.	0.98		0.25	0.77	0.69	0.94	0.21	0.29
		N	21	21	22	22	22		19	22	22	22	21	21
	DHEAS	r	-0.38	0.27	0.01	0.01	1		0.35	-0.25	0.53	0.56	0.40	-0.36
		P	0.09	0.23	0.97	0.98	.		0.14	0.26	0.01	0.01	0.07	0.11
		N	21	21	22	22	22		19	22	22	22	21	21
	E2		Not available for Control Boys										.	.
	Leptin	r	-0.59	0.77	0.11	0.28	0.35		1	-0.52	0.42	0.66	0.36	-0.53
		P	0.01	0.00	0.64	0.25	0.14		.	0.02	0.07	0.00	0.13	0.02
		N	19	19	20	19	19		20	19	20	19	19	19
	SHBG	r	0.27	-0.62	-0.31	0.07	-0.25		-0.52	1	-0.14	-0.57	-0.32	0.22
		P	0.23	0.00	0.16	0.77	0.26		0.02	.	0.55	0.01	0.15	0.34
		N	21	21	22	22	22		19	22	22	22	21	21
	Testos	r	-0.19	0.21	0.29	0.09	0.53		0.42	-0.14	1	0.88	0.31	-0.50
		P	0.40	0.36	0.18	0.69	0.01		0.07	0.55	.	0.00	0.16	0.02
		N	22	22	23	22	22		20	22	23	22	22	22
	FAI	r	-0.27	0.63	0.46	0.02	0.56		0.66	-0.57	0.88	1	0.44	-0.49
		P	0.23	0.00	0.03	0.94	0.01		0.00	0.01	0.00	.	0.05	0.02
		N	21	21	22	22	22		19	22	22	22	21	21
	Age G2	r	-0.53	0.13	0.05	-0.29	0.40		0.36	-0.32	0.31	0.44	1	-0.36
		P	0.01	0.55	0.83	0.21	0.07		0.13	0.15	0.16	0.05	.	0.09
		N	23	23	22	21	21		19	21	22	21	23	23
	Dur G2_PHV	r	0.74	-0.33	-0.36	0.24	-0.36		-0.53	0.22	-0.50	-0.49	-0.36	1
		P	0.00	0.13	0.10	0.29	0.11		0.02	0.34	0.02	0.02	0.09	.
		N	23	23	22	21	21		19	21	22	21	23	23

Correlation Matrix for HtSDS change and Hormones averaged from B2-PHV: Control Girls

			HtSDSΔ PHV-B2	BMI SDS_B2	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI	Age B2	Dur B2_PHV
Control Girls	HtSDSΔ PHV-B2	r	1	0.46	0.18	-0.01	0.39	-0.37	0.46	-0.43	-0.24	-0.04	-0.73	0.81
		P	.	0.01	0.41	0.97	0.14	0.09	0.07	0.10	0.28	0.89	0.00	0.00
		N	31	31	22	16	16	22	16	16	22	16	31	30
	BMISDS_B2	r	0.46	1	0.12	-0.10	0.35	-0.09	0.53	-0.44	-0.03	0.18	-0.65	0.47
		P	0.01	.	0.61	0.73	0.19	0.68	0.04	0.09	0.91	0.51	0.00	0.01
		N	31	31	22	16	16	22	16	16	22	16	31	30
	IGF-I	r	0.18	0.12	1	0.04	0.28	0.16	0.14	-0.14	0.18	0.31	-0.03	-0.22
		P	0.41	0.61	.	0.87	0.24	0.45	0.56	0.56	0.37	0.18	0.90	0.33
		N	22	22	27	20	20	26	20	20	27	20	23	22
	A4	r	-0.01	-0.10	0.04	1	0.71	0.29	0.07	0.15	0.68	0.60	0.10	0.17
		P	0.97	0.73	0.87	.	0.00	0.23	0.79	0.53	0.00	0.01	0.72	0.51
		N	16	16	20	20	20	19	16	20	20	20	17	17
	DHEAS	r	0.39	0.35	0.28	0.71	1	0.33	0.51	-0.26	0.44	0.53	-0.38	0.39
		P	0.14	0.19	0.24	0.00	.	0.17	0.05	0.27	0.05	0.02	0.14	0.12
		N	16	16	20	20	20	19	16	20	20	20	17	17
	E2	r	-0.37	-0.09	0.16	0.29	0.33	1	-0.17	0.11	0.34	0.20	0.42	-0.32
		P	0.09	0.68	0.45	0.23	0.17	.	0.49	0.65	0.09	0.41	0.05	0.14
		N	22	22	26	19	19	26	19	19	26	19	23	22
	Leptin	r	0.46	0.53	0.14	0.07	0.51	-0.17	1	-0.46	-0.05	0.16	-0.42	0.55
		P	0.07	0.04	0.56	0.79	0.05	0.49	.	0.07	0.83	0.54	0.11	0.03
		N	16	16	20	16	16	19	20	16	20	16	16	16
	SHBG	r	-0.43	-0.44	-0.14	0.15	-0.26	0.11	-0.46	1	0.14	-0.27	0.15	-0.35
		P	0.10	0.09	0.56	0.53	0.27	0.65	0.07	.	0.54	0.24	0.56	0.16
		N	16	16	20	20	20	19	16	20	20	20	17	17
	Testos	r	-0.24	-0.03	0.18	0.68	0.44	0.34	-0.05	0.14	1	0.90	0.14	-0.23
		P	0.28	0.91	0.37	0.00	0.05	0.09	0.83	0.54	.	0.00	0.53	0.30
		N	22	22	27	20	20	26	20	20	27	20	23	22
	FAI	r	-0.04	0.18	0.31	0.60	0.53	0.20	0.16	-0.27	0.90	1	0.00	-0.01
		P	0.89	0.51	0.18	0.01	0.02	0.41	0.54	0.24	0.00	.	0.99	0.96
		N	16	16	20	20	20	19	16	20	20	20	17	17
	Age B2	r	-0.73	-0.65	-0.03	0.10	-0.38	0.42	-0.42	0.15	0.14	0.00	1	-0.63
		P	0.00	0.00	0.90	0.72	0.14	0.05	0.11	0.56	0.53	0.99	.	0.00
		N	31	31	23	17	17	23	16	17	23	17	32	31
	Dur B2_PHV	r	0.81	0.47	-0.22	0.17	0.39	-0.32	0.55	-0.35	-0.23	-0.01	-0.63	1
		P	0.00	0.01	0.33	0.51	0.12	0.14	0.03	0.16	0.30	0.96	0.00	.
		N	30	30	22	17	17	22	16	17	22	17	31	31

Correlation Matrix for HtSDS change and Hormones averaged from G2-PHV: T1D Boys

			HtSDSΔ PHV-G2	BMI SDS _G2	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI	Age G2	Dur G2_PHV
T1D Boys	HtSDSΔ PHV-G2	r	1	-0.24	-0.11	0.01	-0.12	0.25	0.10	0.46	-0.41	-0.43	0.06	0.76
		P	.	0.29	0.62	0.97	0.60	0.26	0.73	0.03	0.06	0.05	0.79	0.00
		N	22	22	22	22	22	22	15	22	22	22	22	22
	BMI SDS _G2	r	-0.24	1	0.27	-0.13	0.29	0.31	0.54	-0.50	-0.17	0.04	0.38	-0.10
		P	0.29	.	0.22	0.55	0.18	0.15	0.03	0.01	0.43	0.84	0.08	0.65
		N	22	23	23	23	23	23	16	23	23	23	23	23
	IGF-I	r	-0.11	0.27	1	0.21	0.08	-0.03	0.22	0.02	0.24	0.18	0.12	-0.30
		P	0.62	0.22	.	0.34	0.72	0.90	0.41	0.94	0.28	0.42	0.59	0.17
		N	22	23	23	23	23	23	16	23	23	23	23	23
	A4	r	0.01	-0.13	0.21	1	0.43	0.13	-0.18	-0.37	0.37	0.52	-0.11	-0.27
		P	0.97	0.55	0.34	.	0.04	0.56	0.50	0.09	0.08	0.01	0.62	0.22
		N	22	23	23	23	23	23	16	23	23	23	23	23
	DHEAS	r	-0.12	0.29	0.08	0.43	1	0.05	-0.07	-0.38	0.08	0.26	-0.37	-0.11
		P	0.60	0.18	0.72	0.04	.	0.80	0.80	0.07	0.72	0.23	0.09	0.63
		N	22	23	23	23	23	23	16	23	23	23	23	23
	E2	r	0.25	0.31	-0.03	0.13	0.05	1	0.28	-0.13	0.01	0.06	0.04	0.12
		P	0.26	0.15	0.90	0.56	0.80	.	0.29	0.56	0.96	0.77	0.86	0.59
		N	22	23	23	23	23	23	16	23	23	23	23	23
	Leptin	r	0.10	0.54	0.22	-0.18	-0.07	0.28	1	-0.39	-0.38	-0.20	0.01	0.33
		P	0.73	0.03	0.41	0.50	0.80	0.29	.	0.13	0.15	0.46	0.97	0.22
		N	15	16	16	16	16	16	16	16	16	16	16	16
	SHBG	r	0.46	-0.50	0.02	-0.37	-0.38	-0.13	0.39	1	-0.23	-0.53	0.19	0.35
		P	0.03	0.01	0.94	0.09	0.07	0.56	0.13	.	0.29	0.01	0.39	0.10
		N	22	23	23	23	23	23	16	23	23	23	23	23
	Testos	r	-0.41	-0.17	0.24	0.37	0.08	0.01	0.38	-0.23	1	0.91	0.12	-0.68
		P	0.06	0.43	0.28	0.08	0.72	0.96	0.15	0.29	.	0.00	0.59	0.00
		N	22	23	23	23	23	23	16	23	23	23	23	23
	FAI	r	-0.43	0.04	0.18	0.52	0.26	0.06	0.20	-0.53	0.91	1	0.06	-0.69
		P	0.05	0.84	0.42	0.01	0.23	0.77	0.46	0.01	0.00	.	0.78	0.00
		N	22	23	23	23	23	23	16	23	23	23	23	23
	Age G2	r	-0.06	-0.38	-0.12	-0.11	-0.37	0.04	0.01	0.19	0.12	-0.06	1	0.09
		P	0.79	0.08	0.59	0.62	0.09	0.86	0.97	0.39	0.59	0.78	.	0.68
		N	22	23	23	23	23	23	16	23	23	23	23	23
	Dur G2_PHV	r	0.76	-0.10	-0.30	-0.27	-0.11	0.12	0.33	0.35	-0.68	-0.69	0.09	1
		P	0.00	0.65	0.17	0.22	0.63	0.59	0.22	0.10	0.00	0.00	0.68	.
		N	22	23	23	23	23	23	16	23	23	23	23	23
	HbA1c at G2	r	-0.62	0.00	-0.31	0.10	0.34	-0.07	0.17	-0.41	0.27	0.32	0.10	-0.44
		P	0.00	1.00	0.15	0.65	0.11	0.75	0.52	0.05	0.22	0.14	0.64	0.04
		N	22	23	23	23	23	23	16	23	23	23	23	23
	IN_KG_G2	r	-0.29	0.39	-0.17	-0.19	0.11	0.19	0.43	-0.36	-0.02	0.00	0.05	-0.08
		P	0.19	0.07	0.44	0.39	0.62	0.38	0.09	0.09	0.91	0.99	0.82	0.72
		N	22	23	23	23	23	23	16	23	23	23	23	23
	BA_G2	r	-0.13	-0.06	-0.01	-0.19	-0.01	0.17	0.16	0.02	-0.07	-0.10	0.64	0.07
		P	0.61	0.83	0.98	0.46	0.97	0.51	0.58	0.94	0.79	0.68	0.00	0.80
		N	17	18	18	18	18	18	14	18	18	18	18	18

Correlation Matrix for HtSDS change and Hormones averaged from B2-PHV: T1D Girls

T1D Girls			HtSDS Δ PHV-B2	BMI SDS _B2	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI	Age B2	Dur B2_PHV
	HtSDS Δ PHV-B2	r	1	0.04	-0.34	0.19	0.16	0.53	0.13	0.00	0.30	0.37	-0.75	0.85
		P	.	0.86	0.14	0.43	0.51	0.02	0.70	0.99	0.19	0.12	0.00	0.00
		N	21	21	20	19	19	20	11	19	20	19	21	21
	BMISDS_B2	r	0.04	1	-0.11	0.35	0.13	0.12	0.75	0.03	-0.20	0.00	0.00	0.15
		P	0.86	.	0.66	0.14	0.60	0.62	0.01	0.89	0.40	1.00	0.99	0.52
		N	21	21	20	19	19	20	11	19	20	19	21	21
	IGF-I	r	-0.34	-0.11	1	-0.30	0.14	-0.22	0.00	-0.05	0.21	0.13	0.21	-0.57
		P	0.14	0.66	.	0.20	0.56	0.34	0.99	0.84	0.36	0.58	0.37	0.01
		N	20	20	21	20	20	21	12	20	21	20	20	20
	A4	r	0.19	0.35	-0.30	1	0.06	0.12	0.29	0.11	0.21	0.11	-0.20	0.11
		P	0.43	0.14	0.20	.	0.82	0.60	0.35	0.65	0.38	0.64	0.40	0.66
		N	19	19	20	20	20	20	12	20	20	20	19	19
	DHEAS	r	0.16	0.13	0.14	0.06	1	-0.08	-0.19	0.00	0.47	0.30	-0.19	0.07
		P	0.51	0.60	0.56	0.82	.	0.74	0.56	1.00	0.04	0.20	0.43	0.77
		N	19	19	20	20	20	20	12	20	20	20	19	19
	E2	r	0.53	0.12	-0.22	0.12	-0.08	1	0.12	-0.07	0.10	0.13	0.37	0.51
		P	0.02	0.62	0.34	0.60	0.74	.	0.71	0.78	0.65	0.58	0.11	0.02
		N	20	20	21	20	20	21	12	20	21	20	20	20
	Leptin	r	0.13	0.75	0.00	0.29	-0.19	0.12	1	-0.18	0.15	0.21	0.26	0.08
		P	0.70	0.01	0.99	0.35	0.56	0.71	.	0.58	0.65	0.52	0.45	0.81
		N	11	11	12	12	12	12	12	12	12	12	11	11
	SHBG	r	0.00	0.03	-0.05	0.11	0.00	-0.07	-0.18	1	-0.32	-0.71	0.11	-0.07
		P	0.99	0.89	0.84	0.65	1.00	0.78	0.58	.	0.17	0.00	0.65	0.79
		N	19	19	20	20	20	20	12	20	20	20	19	19
	Testos	r	0.30	-0.20	0.21	0.21	0.47	0.10	0.15	-0.32	1	0.81	-0.30	0.21
		P	0.19	0.40	0.36	0.38	0.04	0.65	0.65	0.17	.	0.00	0.19	0.38
		N	20	20	21	20	20	21	12	20	21	20	20	20
	FAI	r	0.37	0.00	0.13	0.11	0.30	0.13	0.21	-0.71	0.81	1	-0.35	0.30
		P	0.12	1.00	0.58	0.64	0.20	0.58	0.52	0.00	0.00	.	0.14	0.22
		N	19	19	20	20	20	20	12	20	20	20	19	19
	Age B2	r	-0.75	0.00	0.21	-0.20	-0.19	-0.37	-0.26	0.11	-0.30	-0.35	1	-0.58
		P	0.00	0.99	0.37	0.40	0.43	0.11	0.45	0.65	0.19	0.14	.	0.01
		N	21	21	20	19	19	20	11	19	20	19	21	21
	Dur B2_PHV	r	0.85	0.15	-0.57	0.11	0.07	0.51	0.08	-0.07	0.21	0.30	-0.58	1
		P	0.00	0.52	0.01	0.66	0.77	0.02	0.81	0.79	0.38	0.22	0.01	.
		N	21	21	20	19	19	20	11	19	20	19	21	21
	HbA1c at B2	r	-0.45	0.08	0.27	-0.28	-0.19	-0.16	-0.22	0.02	-0.56	-0.56	0.53	-0.34
		P	0.04	0.72	0.24	0.24	0.43	0.49	0.51	0.92	0.01	0.01	0.01	0.13
		N	21	21	20	19	19	20	11	19	20	19	21	21
	IN_KG_B2	r	-0.55	-0.31	0.35	-0.28	-0.14	-0.12	-0.44	-0.16	-0.03	-0.26	0.47	-0.48
		P	0.01	0.17	0.13	0.25	0.57	0.61	0.17	0.51	0.91	0.29	0.03	0.03
		N	21	21	20	19	19	20	11	19	20	19	21	21
	BA_B2	r	-0.31	0.43	0.23	-0.25	0.37	0.18	0.35	-0.50	0.31	0.44	0.36	-0.12
		P	0.23	0.09	0.40	0.34	0.16	0.51	0.40	0.05	0.24	0.09	0.16	0.66
		N	17	17	16	16	16	16	8	16	16	16	17	17

8.13.4 PHVSDS and Hormones at PHV

Correlation Matrix for PHVSDS and Hormones at PHV: All Boys

		PHVSDS	BMI_SDS at PHV	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI
PHVSDS	r	1	-0.16	0.29	0.43	-0.05		-0.17	0.16	0.30	0.15
	p		0.27	0.05	0.00	0.74		0.33	0.30	0.04	0.34
	N	46	46	45	44	44		34	44	46	44
BMI_SDS at PHV	r	-0.16	1	-0.11	-0.24	0.08		0.77	-0.22	-0.22	0.03
	p	0.27		0.46	0.12	0.63		0.00	0.15	0.15	0.86
	N	46	47	45	44	44		34	44	46	44
IGF-I	r	0.29	-0.11	1	0.11	0.07		-0.27	-0.25	0.28	0.33
	p	0.05	0.46		0.48	0.66		0.12	0.11	0.06	0.03
	N	45	45	45	43	43		34	43	45	43
A4	r	0.43	-0.24	0.11	1	0.24		-0.30	-0.18	0.40	0.37
	p	0.00	0.12	0.48		0.11		0.10	0.25	0.01	0.01
	N	44	44	43	44	44		32	44	44	44
DHEAS	r	-0.05	0.08	0.07	0.24	1		0.03	-0.39	0.26	0.35
	p	0.74	0.63	0.66	0.11			0.88	0.01	0.09	0.02
	N	44	44	43	44	44		32	44	44	44
E2					Not available for Control Boys						
Leptin	r	-0.17	0.77	-0.27	-0.30	0.03		1	-0.10	-0.24	-0.10
	p	0.33	0.00	0.12	0.10	0.88			0.58	0.17	0.57
	N	34	34	34	32	32		34	32	34	32
SHBG	r	0.16	-0.22	-0.25	-0.18	-0.39		-0.10	1	-0.29	-0.67
	p	0.30	0.15	0.11	0.25	0.01		0.58		0.06	0.00
	N	44	44	43	44	44		32	44	44	44
Testos	r	0.30	-0.22	0.28	0.40	0.26		-0.24	-0.29	1	0.88
	p	0.04	0.15	0.06	0.01	0.09		0.17	0.06		0.00
	N	46	46	45	44	44		34	44	46	44
FAI	r	0.15	0.03	0.33	0.37	0.35		-0.10	-0.67	0.88	1
	p	0.34	0.86	0.03	0.01	0.02		0.57	0.00	0.00	
	N	44	44	43	44	44		32	44	44	44

Correlation Matrix of PHVSDS and Hormones at PHV: T1D and Control Boys

			PHVSDS	BMI_SDS at PHV	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI
T1D Boys	PHVSDS	r	1	-0.12	0.42	0.41	-0.07	0.32	0.02	0.06	0.10	0.05
		p		0.60	0.05	0.06	0.75	0.14	0.93	0.78	0.64	0.84
		N	23	23	22	22	22	22	14	22	23	22
	BMI_SDS at PHV	r	-0.12	1	0.00	-0.05	0.46	0.25	0.46	-0.35	0.13	0.27
		p	0.60		1.00	0.83	0.03	0.26	0.10	0.11	0.57	0.22
		N	23	24	22	22	22	22	14	22	23	22
	IGF-I	r	0.42	0.00	1	0.00	0.00	0.35	0.13	-0.09	0.16	0.16
		p	0.05	1.00		0.98	0.99	0.12	0.67	0.69	0.46	0.48
		N	22	22	22	21	21	21	14	21	22	21
	A4	r	0.41	-0.05	0.00	1	0.44	0.20	-	-0.54	0.26	0.42
		p	0.06	0.83	0.98		0.04	0.40	0.98	0.01	0.25	0.05
		N	22	22	21	22	22	21	13	22	22	22
	DHEAS	r	-0.07	0.46	0.00	0.44	1	-0.01	0.42	-0.46	0.06	0.23
		p	0.75	0.03	0.99	0.04		0.97	0.16	0.03	0.80	0.31
		N	22	22	21	22	22	21	13	22	22	22
	E2	r	0.32	0.25	0.35	0.20	-0.01	1	0.13	-0.26	0.19	0.26
		p	0.14	0.26	0.12	0.40	0.97		0.66	0.25	0.40	0.26
		N	22	22	21	21	21	22	14	21	22	21
	Leptin	r	0.02	0.46	0.13	-0.01	0.42	0.13	1	-0.39	0.29	0.37
		p	0.93	0.10	0.67	0.98	0.16	0.66		0.18	0.32	0.21
		N	14	14	14	13	13	14	14	13	14	13
	SHBG	r	0.06	-0.35	-0.09	-0.54	-0.46	-0.26	-	1	-0.21	-0.57
		p	0.78	0.11	0.69	0.01	0.03	0.25	0.18		0.35	0.01
		N	22	22	21	22	22	21	13	22	22	22
	Testos	r	0.10	0.13	0.16	0.26	0.06	0.19	0.29	-0.21	1	0.90
		p	0.64	0.57	0.46	0.25	0.80	0.40	0.32	0.35		0.00
		N	23	23	22	22	22	22	14	22	23	22
	FAI	r	0.05	0.27	0.16	0.42	0.23	0.26	0.37	-0.57	0.90	1
		p	0.84	0.22	0.48	0.05	0.31	0.26	0.21	0.01	0.00	
		N	22	22	21	22	22	21	13	22	22	22
Control Boys	PHVSDS	r	1	-0.14	0.14	0.40	-0.17		-	0.40	0.53	0.12
		p		0.51	0.51	0.06	0.44		0.45	0.06	0.01	0.58
		N	23	23	23	22	22		20	22	23	22
	BMI_SDS at PHV	r	-0.14	1	0.13	-0.19	0.08		0.82	-0.50	-0.19	0.32
		p	0.51		0.55	0.40	0.72		0.00	0.02	0.39	0.14
		N	23	23	23	22	22		20	22	23	22
	IGF-I	r	0.14	0.13	1	-0.10	-0.40		-	0.03	-0.09	-0.08
		p	0.51	0.55		0.66	0.07		0.29	0.88	0.68	0.74
		N	23	23	23	22	22		20	22	23	22
	A4	r	0.40	-0.19	-0.10	1	-0.04		-	0.22	0.40	0.16
		p	0.06	0.40	0.66		0.86		0.94	0.33	0.07	0.48
		N	22	22	22	22	22		19	22	22	22
	DHEAS	r	-0.17	0.08	-0.40	-0.04	1		0.16	-0.09	0.13	0.13
		p	0.44	0.72	0.07	0.86			0.53	0.70	0.55	0.57
		N	22	22	22	22	22		19	22	22	22
	E2	Not available for Control Boys										
	Leptin	r	-0.18	0.82	-0.25	-0.02	0.16		1	-0.43	-0.07	0.32
		p	0.45	0.00	0.29	0.94	0.53			0.07	0.78	0.18
		N	20	20	20	19	19		20	19	20	19
	SHBG	r	0.40	-0.50	0.03	0.22	-0.09		-	1	0.04	-0.64
		p	0.06	0.02	0.88	0.33	0.70		0.07		0.87	0.00
		N	22	22	22	22	22		19	22	22	22
	Testos	r	0.53	-0.19	-0.09	0.40	0.13		-	0.04	1	0.70
		p	0.01	0.39	0.68	0.07	0.55		0.78	0.87		0.00
		N	23	23	23	22	22		20	22	23	22
	FAI	r	0.12	0.32	-0.08	0.16	0.13		0.32	-0.64	0.70	1
		p	0.58	0.14	0.74	0.48	0.57		0.18	0.00	0.00	
		N	22	22	22	22	22		19	22	22	22

Correlation Matrix for PHVSDS and Hormones at PHV: All Girls

		PHVSDS	BMI_SDS at PHV	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI
PHVSDS	r	1	-0.33	-0.07	0.18	-0.11	0.05	-0.26	0.22	0.02	-0.06
	p		0.02	0.62	0.27	0.52	0.72	0.16	0.19	0.92	0.74
	N	52	52	47	38	38	46	31	38	47	37
BMI_SDS at PHV	r	-0.33	1	-0.07	-0.09	0.02	-0.03	0.73	-0.20	0.04	0.11
	p	0.02		0.64	0.60	0.93	0.86	0.00	0.23	0.82	0.52
	N	52	52	47	38	38	46	31	38	47	37
IGF-I	r	-0.07	-0.07	1	0.09	0.44	0.15	-0.14	-0.15	0.27	0.28
	p	0.62	0.64		0.61	0.01	0.33	0.47	0.38	0.07	0.09
	N	47	47	47	37	37	45	31	37	46	37
A4	r	0.18	-0.09	0.09	1	0.47	0.42	-0.20	-0.10	0.46	0.42
	p	0.27	0.60	0.61		0.00	0.01	0.31	0.55	0.00	0.01
	N	38	38	37	38	38	37	27	38	38	37
DHEAS	r	-0.11	0.02	0.44	0.47	1	0.37	-0.33	-0.18	0.48	0.51
	p	0.52	0.93	0.01	0.00		0.02	0.09	0.29	0.00	0.00
	N	38	38	37	38	38	37	27	38	38	37
E2	r	0.05	-0.03	0.15	0.42	0.37	1	-0.16	-0.12	0.42	0.42
	p	0.72	0.86	0.33	0.01	0.02		0.41	0.47	0.00	0.01
	N	46	46	45	37	37	46	30	37	46	36
Leptin	r	-0.26	0.73	-0.14	-0.20	-0.33	-0.16	1	0.05	-0.14	-0.16
	p	0.16	0.00	0.47	0.31	0.09	0.41		0.82	0.44	0.42
	N	31	31	31	27	27	30	31	27	31	27
SHBG	r	0.22	-0.20	-0.15	-0.10	-0.18	-0.12	0.05	1	-0.15	-0.48
	p	0.19	0.23	0.38	0.55	0.29	0.47	0.82		0.38	0.00
	N	38	38	37	38	38	37	27	38	38	37
Testos	r	0.02	0.04	0.27	0.46	0.48	0.42	-0.14	-0.15	1	0.86
	p	0.92	0.82	0.07	0.00	0.00	0.00	0.44	0.38		0.00
	N	47	47	46	38	38	46	31	38	47	37
FAI	r	-0.06	0.11	0.28	0.42	0.51	0.42	-0.16	-0.48	0.86	1
	p	0.74	0.52	0.09	0.01	0.00	0.01	0.42	0.00	0.00	
	N	37	37	37	37	37	36	27	37	37	37

Correlation Matrix of PHVSDS and Hormones at PHV: T1D and Control Girls

			PHVSDS	BMI_SDS at PHV	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI
T1D Girls	PHVSDS	r	1	-0.45	-0.13	0.21	-0.29	0.08	-0.16	0.36	-0.06	-0.28
		p		0.04	0.59	0.41	0.24	0.74	0.64	0.15	0.79	0.28
		N	22	22	20	18	18	20	11	18	20	17
	BMI_SDS at PHV	r	-0.45	1	-0.15	0.22	0.07	0.12	0.81	-0.10	-0.10	0.25
		p	0.04		0.53	0.39	0.78	0.60	0.00	0.70	0.66	0.33
		N	22	22	20	18	18	20	11	18	20	17
	IGF-I	r	-0.13	-0.15	1	-0.25	0.31	-0.12	0.05	-0.01	0.26	0.09
		p	0.59	0.53	.	0.33	0.22	0.63	0.90	0.97	0.28	0.72
		N	20	20	20	17	17	19	11	17	19	17
	A4	r	0.21	0.22	-0.25	1	0.07	0.33	0.52	0.04	0.30	0.02
		p	0.41	0.39	0.33	.	0.79	0.18	0.10	0.89	0.23	0.95
		N	18	18	17	18	18	18	11	18	18	17
	DHEAS	r	-0.29	0.07	0.31	0.07	1	0.05	-0.23	0.00	0.51	0.34
		p	0.24	0.78	0.22	0.79	.	0.85	0.50	1.00	0.03	0.18
		N	18	18	17	18	18	18	11	18	18	17
	E2	r	0.08	0.12	-0.12	0.33	0.05	1	0.28	-0.16	0.30	0.25
		p	0.74	0.60	0.63	0.18	0.85	.	0.41	0.54	0.20	0.34
		N	20	20	19	18	18	20	11	18	20	17
	Leptin	r	-0.16	0.81	0.05	0.52	-0.23	0.28	1	-0.06	0.09	0.10
		p	0.64	0.00	0.90	0.10	0.50	0.41	.	0.87	0.80	0.76
		N	11	11	11	11	11	11	11	11	11	11
	SHBG	r	0.36	-0.10	-0.01	0.04	0.00	-0.16	-0.06	1	-0.36	-0.60
		p	0.15	0.70	0.97	0.89	1.00	0.54	0.87	.	0.14	0.01
		N	18	18	17	18	18	18	11	18	18	17
	Testos	r	-0.06	-0.10	0.26	0.30	0.51	0.30	0.09	-0.36	1	0.82
		p	0.79	0.66	0.28	0.23	0.03	0.20	0.80	0.14	.	0.00
		N	20	20	19	18	18	20	11	18	20	17
	FAI	r	-0.28	0.25	0.09	0.02	0.34	0.25	0.10	-0.60	0.82	1
		p	0.28	0.33	0.72	0.95	0.18	0.34	0.76	0.01	0.00	.
		N	17	17	17	17	17	17	11	17	17	17
Control Girls	PHVSDS	r	1	-0.23	-0.11	0.02	-0.02	-0.02	-0.11	0.07	0.05	0.09
		p		0.21	0.57	0.95	0.94	0.94	0.64	0.77	0.80	0.70
		N	30	30	27	20	20	26	20	20	27	20
	BMI_SDS at PHV	r	-0.23	1	0.09	0.06	0.39	0.01	0.72	-0.43	0.15	0.27
		p	0.21		0.65	0.79	0.09	0.97	0.00	0.06	0.45	0.24
		N	30	30	27	20	20	26	20	20	27	20
	IGF-I	r	-0.11	0.09	1	0.25	0.52	0.17	0.11	-0.22	0.21	0.37
		p	0.57	0.65		0.29	0.02	0.42	0.64	0.36	0.29	0.11
		N	27	27	27	20	20	26	20	20	27	20
	A4	r	0.02	0.06	0.25	1	0.75	0.41	0.14	-0.16	0.68	0.67
		p	0.95	0.79	0.29		0.00	0.08	0.61	0.51	0.00	0.00
		N	20	20	20	20	20	19	16	20	20	20
	DHEAS	r	-0.02	0.39	0.52	0.75	1	0.54	0.51	-0.32	0.48	0.57
		p	0.94	0.09	0.02	0.00		0.02	0.04	0.16	0.03	0.01
		N	20	20	20	20	20	19	16	20	20	20
	E2	r	-0.02	0.01	0.17	0.41	0.54	1	-0.11	-0.05	0.47	0.45
		p	0.94	0.97	0.42	0.08	0.02		0.67	0.83	0.02	0.05
		N	26	26	26	19	19	26	19	19	26	19
	Leptin	r	-0.11	0.72	0.11	0.14	0.51	-0.11	1	-0.33	0.11	0.27
		p	0.64	0.00	0.64	0.61	0.04	0.67		0.21	0.64	0.32
		N	20	20	20	16	16	19	20	16	20	16
	SHBG	r	0.07	-0.43	-0.22	-0.16	-0.32	-0.05	-0.33	1	0.05	-0.32
		p	0.77	0.06	0.36	0.51	0.16	0.83	0.21		0.84	0.17
		N	20	20	20	20	20	19	16	20	20	20
	Testos	r	0.05	0.15	0.21	0.68	0.48	0.47	0.11	0.05	1	0.90
		p	0.80	0.45	0.29	0.00	0.03	0.02	0.64	0.84		0.00
		N	27	27	27	20	20	26	20	20	27	20
	FAI	r	0.09	0.27	0.37	0.67	0.57	0.45	0.27	-0.32	0.90	1
		p	0.70	0.24	0.11	0.00	0.01	0.05	0.32	0.17	0.00	
		N	20	20	20	20	20	19	16	20	20	20

8.13.5 PHVSDS and Hormones averaged from Stage 2-PHV

Correlation Matrix for PHVSDS and Hormones averaged from G2 to PHV: All Boys

			PHVSDS	BMI_SDS at G2	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI
All Boys	PHVSDS	r	1	-0.18	0.10	0.22	-0.17	0.37	-0.15	0.32	0.14	0.02
		p		0.23	0.51	0.15	0.28	0.08	0.40	0.03	0.37	0.89
		N	46	45	45	44	44	23	36	44	45	44
	BMI_SDS at G2	r	-0.18	1	0.19	-0.13	0.16	0.31	0.74	-0.39	-0.07	0.15
		p	0.23		0.21	0.41	0.31	0.15	0.00	0.01	0.65	0.34
		N	45	45	44	43	43	23	35	43	44	43
	IGF-I	r	0.10	0.19	1	-0.09	0.07	-0.03	-0.01	-0.18	0.28	0.30
		p	0.51	0.21		0.54	0.63	0.90	0.96	0.23	0.07	0.05
		N	45	44	45	44	44	23	36	44	45	44
	A4	r	0.22	-0.13	-0.09	1	0.24	0.13	-0.09	-0.15	0.24	0.29
		p	0.15	0.41	0.54		0.12	0.56	0.60	0.32	0.11	0.06
		N	44	43	44	44	44	23	35	44	44	44
	DHEAS	r	-0.17	0.16	0.07	0.24	1	0.05	0.03	-0.33	0.26	0.37
		p	0.28	0.31	0.63	0.12		0.80	0.85	0.03	0.09	0.01
		N	44	43	44	44	44	23	35	44	44	44
	E2		Not available for Control Boys									
	Leptin	r	-0.15	0.74	-0.01	-0.09	0.03	0.28	1	-0.12	-0.20	-0.13
		p	0.40	0.00	0.96	0.60	0.85	0.29		0.50	0.25	0.44
		N	36	35	36	35	35	16	36	35	36	35
	SHBG	r	0.32	-0.39	-0.18	-0.15	-0.33	-0.13	-0.12	1	-0.20	-0.54
		p	0.03	0.01	0.23	0.32	0.03	0.56	0.50		0.20	0.00
		N	44	43	44	44	44	23	35	44	44	44
	Testos	r	0.14	-0.07	0.28	0.24	0.26	0.01	-0.20	-0.20	1	0.90
		p	0.37	0.65	0.07	0.11	0.09	0.96	0.25	0.20		0.00
		N	45	44	45	44	44	23	36	44	45	44
	FAI	r	0.02	0.15	0.30	0.29	0.37	0.06	-0.13	-0.54	0.90	1
		p	0.89	0.34	0.05	0.06	0.01	0.77	0.44	0.00	0.00	
		N	44	43	44	44	44	23	35	44	44	44

Correlation Matrix of PHVSDS and Hormones averaged Stage 2-PHV: T1D and Control Boys

			PHVSDS	BMI_SDS at G2	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI
T1D Boys	PHVSD	r	1	-0.09	0.23	0.32	-0.25	0.37	0.19	0.19	0.10	0.03
		p		0.68	0.28	0.14	0.25	0.08	0.47	0.38	0.63	0.90
		N	23	23	23	23	23	23	16	23	23	23
	BMI_SDS at G2	r	-0.09	1	0.27	-0.29	0.31	0.54	-0.50	-0.17	0.04	
		p	0.68		0.22	0.55	0.18	0.15	0.03	0.01	0.43	0.84
		N	23	23	23	23	23	23	16	23	23	23
	IGF-I	r	0.23	0.27	1	0.21	0.08	-0.03	0.22	0.02	0.24	0.18
		p	0.28	0.22		0.34	0.72	0.90	0.41	0.94	0.28	0.42
		N	23	23	23	23	23	23	16	23	23	23
	A4	r	0.32	-0.13	0.21	1	0.43	0.13	-0.18	-0.37	0.37	0.52
		p	0.14	0.55	0.34		0.04	0.56	0.50	0.09	0.08	0.01
		N	23	23	23	23	23	23	16	23	23	23
	DHEAS	r	-0.25	0.29	0.08	0.43	1	0.05	-0.07	-0.38	0.08	0.26
		p	0.25	0.18	0.72	0.04		0.80	0.80	0.07	0.72	0.23
		N	23	23	23	23	23	23	16	23	23	23
	E2	r	0.37	0.31	-0.03	0.13	0.05	1	0.28	-0.13	0.01	0.06
		p	0.08	0.15	0.90	0.56	0.80		0.29	0.56	0.96	0.77
		N	23	23	23	23	23	23	16	23	23	23
	Leptin	r	0.19	0.54	0.22	-0.50	-0.07	0.28	1	-0.39	-0.38	-
		p	0.47	0.03	0.41	0.50	0.80	0.29		0.13	0.15	0.46
		N	16	16	16	16	16	16	16	16	16	16
	SHBG	r	0.19	-0.50	0.02	-0.09	-0.38	-0.13	-0.39	1	-0.23	-
		p	0.38	0.01	0.94	0.09	0.07	0.56	0.13		0.29	0.01
		N	23	23	23	23	23	23	16	23	23	23
	Testos	r	0.10	-0.17	0.24	0.37	0.08	0.01	-0.38	-0.23	1	0.91
		p	0.63	0.43	0.28	0.08	0.72	0.96	0.15	0.29		0.00
		N	23	23	23	23	23	23	16	23	23	23
	FAI	r	0.03	0.04	0.18	0.52	0.26	0.06	-0.20	-0.53	0.91	1
		p	0.90	0.84	0.42	0.01	0.23	0.77	0.46	0.01	0.00	
		N	23	23	23	23	23	23	16	23	23	23
Control Boys	PHVSD	r	1	-0.17	-0.10	0.15	-0.17		-0.19	0.57	0.13	-
		p		0.44	0.65	0.51	0.47		0.43	0.01	0.55	0.72
		N	23	22	22	21	21		20	21	22	21
	BMI_SDS at G2	r	-0.17	1	0.38	-0.09	0.22		0.77	-0.59	0.17	0.60
		p	0.44		0.09	0.78	0.36		0.00	0.01	0.46	0.00
		N	22	22	21	20	20		19	20	21	20
	IGF-I	r	-0.10	0.38	1	-0.10	-0.04		0.11	-0.29	0.27	0.44
		p	0.65	0.09		0.10	0.88		0.64	0.20	0.22	0.04
		N	22	21	22	21	21		20	21	22	21
	A4	r	0.15	-0.07	-0.37	1	0.11		0.28	0.00	0.17	0.11
		p	0.51	0.78	0.10		0.63		0.25	0.98	0.47	0.62
		N	21	20	21	21	21		19	21	21	21
	DHEAS	r	-0.17	0.22	-0.04	0.11	1		0.35	-0.19	0.50	0.52
		p	0.47	0.36	0.88	0.63			0.14	0.40	0.02	0.02
		N	21	20	21	21	21		19	21	21	21
	E2	Not available for Control Boys										
	Leptin	r	-0.19	0.77	0.11	0.28	0.35		1	-0.52	0.42	0.66
		p	0.43	0.00	0.64	0.25	0.14			0.02	0.07	0.00
		N	20	19	20	19	19		20	19	20	19
	SHBG	r	0.57	-0.59	-0.29	0.00	-0.19		-0.52	1	-0.09	-
		p	0.01	0.01	0.20	0.98	0.40		0.02		0.69	0.01
		N	21	20	21	21	21		19	21	21	21
	Testos	r	0.13	0.17	0.27	0.17	0.50		0.42	-0.09	1	0.88
		p	0.55	0.46	0.22	0.47	0.02		0.07	0.69		0.00
		N	22	21	22	21	21		20	21	22	21
	FAI	r	-0.08	0.60	0.44	0.11	0.52		0.66	-0.55	0.88	1
		p	0.72	0.00	0.04	0.62	0.02		0.00	0.01	0.00	
		N	21	20	21	21	21		19	21	21	21

Correlation Matrix for PHVSDS and Hormones averaged from B2 to PHV: All Girls

			PHVSDS	BMI_SDS at B2	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI
All Girls	PHVSDS	r	1	-0.31	-0.03	0.21	-0.04	0.18	-0.25	0.18	0.14	0.03
		p		0.02	0.85	0.18	0.81	0.24	0.16	0.25	0.35	0.83
		N	58	52	48	40	40	47	32	40	48	40
	BMI_SDS at B2	r	-0.31	1	-0.04	-0.11	0.05	-	0.64	-0.12	-0.08	0.04
		p	0.02		0.80	0.53	0.78	0.51	0.00	0.51	0.61	0.81
		N	52	52	42	35	35	42	27	35	42	35
	IGF-I	r	-0.03	-0.04	1	-0.05	0.24	0.08	-0.11	-0.10	0.21	0.24
		p	0.85	0.80		0.77	0.14	0.60	0.56	0.54	0.16	0.14
		N	48	42	48	40	40	47	32	40	48	40
	A4	r	0.21	-0.11	-0.05	1	0.47	0.30	-0.31	0.03	0.46	0.43
		p	0.18	0.53	0.77		0.00	0.07	0.11	0.87	0.00	0.01
		N	40	35	40	40	40	39	28	40	40	40
	DHEAS	r	-0.04	0.05	0.24	0.47	1	0.23	-0.32	-0.14	0.44	0.46
		p	0.81	0.78	0.14	0.00		0.15	0.10	0.38	0.00	0.00
		N	40	35	40	40	40	39	28	40	40	40
	E2	r	0.18	-0.10	0.08	0.30	0.23	1	-0.21	0.00	0.29	0.22
		p	0.24	0.51	0.60	0.07	0.15		0.26	0.99	0.04	0.17
		N	47	42	47	39	39	47	31	39	47	39
	Leptin	r	-0.25	0.64	-0.11	-0.31	-0.32	-	1	0.00	-0.19	-
		p	0.16	0.00	0.56	0.11	0.10	0.26		0.98	0.30	0.41
		N	32	27	32	28	28	31	32	28	32	28
	SHBG	r	0.18	-0.12	-0.10	0.03	-0.14	0.00	0.00	1	-0.08	-
		p	0.25	0.51	0.54	0.87	0.38	0.99	0.98		0.64	0.00
		N	40	35	40	40	40	39	28	40	40	40
	Testos	r	0.14	-0.08	0.21	0.46	0.44	0.29	-0.19	-0.08	1	0.86
		p	0.35	0.61	0.16	0.00	0.00	0.04	0.30	0.64		0.00
		N	48	42	48	40	40	47	32	40	48	40
	FAI	r	0.03	0.04	0.24	0.43	0.46	0.22	-0.16	-0.50	0.86	1
		p	0.83	0.81	0.14	0.01	0.00	0.17	0.41	0.00	0.00	
		N	40	35	40	40	40	39	28	40	40	40

Correlation Matrix of PHVSDS and Hormones averaged B2-PHV: T1D and Control Girls

			PHVSDS	BMI_SDS at B2	IGF-I	A4	DHEAS	E2	Leptin	SHBG	Testos	FAI
T1D Girls		r	1	-0.43	-0.21	0.24	-0.20	0.26	-0.16	0.34	0.02	-0.16
	PHVSDS	p		0.05	0.36	0.31	0.40	0.25	0.63	0.14	0.93	0.50
		N	22	21	21	20	20	21	12	20	21	20
		r	-0.43	1	-0.11	0.35	0.13	0.12	0.75	0.03	-0.20	0.00
	BMI_SDS at B2	p	0.05		0.66	0.14	0.60	0.62	0.01	0.89	0.40	1.00
		N	21	21	20	19	19	20	11	19	20	19
	IGF-I	r	-0.21	-0.11	1	-0.30	0.14	-	0.00	-0.05	0.21	0.13
		p	0.36	0.66		0.20	0.56	0.34	0.99	0.84	0.36	0.58
		N	21	20	21	20	20	21	12	20	21	20
	A4	r	0.24	0.35	-0.30	1	0.06	0.12	0.29	0.11	0.21	0.11
		p	0.31	0.14	0.20		0.82	0.60	0.35	0.65	0.38	0.64
		N	20	19	20	20	20	20	12	20	20	20
	DHEAS	r	-0.20	0.13	0.14	0.06	1	-	-0.19	0.00	0.47	0.30
		p	0.40	0.60	0.56	0.82		0.74	0.56	1.00	0.04	0.20
		N	20	19	20	20	20	20	12	20	20	20
	E2	r	0.26	0.12	-0.22	0.12	-0.08	1	0.12	-0.07	0.10	0.13
		p	0.25	0.62	0.34	0.60	0.74		0.71	0.78	0.65	0.58
		N	21	20	21	20	20	21	12	20	21	20
	Leptin	r	-0.16	0.75	0.00	0.29	-0.19	0.12	1	-0.18	0.15	0.21
		p	0.63	0.01	0.99	0.35	0.56	0.71		0.58	0.65	0.52
		N	12	11	12	12	12	12	12	12	12	12
	SHBG	r	0.34	0.03	-0.05	0.11	0.00	-	-0.18	1	-0.32	-0.71
		p	0.14	0.89	0.84	0.65	1.00	0.78	0.58		0.17	0.00
		N	20	19	20	20	20	20	12	20	20	20
	Testos	r	0.02	-0.20	0.21	0.21	0.47	0.10	0.15	-0.32	1	0.81
		p	0.93	0.40	0.36	0.38	0.04	0.65	0.65	0.17		0.00
		N	21	20	21	20	20	21	12	20	21	20
	FAI	r	-0.16	0.00	0.13	0.11	0.30	0.13	0.21	-0.71	0.81	1
		p	0.50	1.00	0.58	0.64	0.20	0.58	0.52	0.00	0.00	
		N	20	19	20	20	20	20	12	20	20	20
Control Girls		r	1	-0.23	0.12	0.10	0.08	0.13	-0.10	-0.03	0.21	0.18
	PHVSDS	p		0.21	0.54	0.67	0.75	0.52	0.68	0.91	0.30	0.44
		N	36	31	27	20	20	26	20	20	27	20
		r	-0.23	1	0.12	-0.10	0.35	-	0.53	-0.44	-0.03	0.18
	BMI_SDS at B2	p	0.21		0.61	0.73	0.19	0.68	0.04	0.09	0.91	0.51
		N	31	31	22	16	16	22	16	16	22	16
	IGF-I	r	0.12	0.12	1	0.04	0.28	0.16	0.14	-0.14	0.18	0.31
		p	0.54	0.61		0.87	0.24	0.45	0.56	0.56	0.37	0.18
		N	27	22	27	20	20	26	20	20	27	20
	A4	r	0.10	-0.10	0.04	1	0.71	0.29	0.07	0.15	0.68	0.60
		p	0.67	0.73	0.87		0.00	0.23	0.79	0.53	0.00	0.01
		N	20	16	20	20	20	19	16	20	20	20
	DHEAS	r	0.08	0.35	0.28	0.71	1	0.33	0.51	-0.26	0.44	0.53
		p	0.75	0.19	0.24	0.00		0.17	0.05	0.27	0.05	0.02
		N	20	16	20	20	20	19	16	20	20	20
	E2	r	0.13	-0.09	0.16	0.29	0.33	1	-0.17	0.11	0.34	0.20
		p	0.52	0.68	0.45	0.23	0.17		0.49	0.65	0.09	0.41
		N	26	22	26	19	19	26	19	19	26	19
	Leptin	r	-0.10	0.53	0.14	0.07	0.51	-	1	-0.46	-0.05	0.16
		p	0.68	0.04	0.56	0.79	0.05	0.49		0.07	0.83	0.54
		N	20	16	20	16	16	19	20	16	20	16
	SHBG	r	-0.03	-0.44	-0.14	0.15	-0.26	0.11	-0.46	1	0.14	-0.27
		p	0.91	0.09	0.56	0.53	0.27	0.65	0.07		0.54	0.24
		N	20	16	20	20	20	19	16	20	20	20
	Testos	r	0.21	-0.03	0.18	0.68	0.44	0.34	-0.05	0.14	1	0.90
		p	0.30	0.91	0.37	0.00	0.05	0.09	0.83	0.54		0.00
		N	27	22	27	20	20	26	20	20	27	20
	FAI	r	0.18	0.18	0.31	0.60	0.53	0.20	0.16	-0.27	0.90	1
		p	0.44	0.51	0.18	0.01	0.02	0.41	0.54	0.24	0.00	
		N	20	16	20	20	20	19	16	20	20	20

Publications arising from these studies

Ahmed ML, Connors MH, Drayer NM, Jones JS, Dunger DB. 1998 Pubertal growth in IDDM is determined by HbA1c levels, sex, and bone age. Diabetes Care. 21:831-835.

Ahmed ML, Ong KK, Morrell DJ et al. 1999 Longitudinal study of leptin concentrations during puberty: sex differences and relationship to changes in body composition. J Clin Endocrinol Metab. 84:899-905.

Ong KKL, Ahmed ML, Dunger DB. 1999 The role of leptin in human growth and puberty. Proceedings of the 27th International Symposium on Growth Hormone and Growth Factors in Endocrinology and Metabolism. Acta Paediatr Supplement 433:95-98.

Ahmed ML, Ong KKL, Watts AP, Morrell DJ, Preece MA, Dunger DB. 2001 Elevated Leptin Levels are Associated with Excess Gains in Fat Mass in Girls, but not Boys with Type 1 Diabetes: Longitudinal Study during Adolescence. J Clin Endocrinol Metab. 86:1188-1193.

Dunger D, Ahmed L, Ong K. 2002 Growth and Body Composition in Type 1 Diabetes Mellitus. Horm Res 58 (suppl 1): 66-71.

Bibliography

Adair LS (2001) Size at birth predicts age at menarche. Pediatrics 107:E59

Adan L, Bussieres L, Trivin C, Souberbielle JC, Brauner R (1999) Effect of short-term testosterone treatment on leptin concentrations in boys with pubertal delay. Horm Res 52:109-12

Ahima RS, Dushay J, Flier SN, Prabakaran D, Flier JS (1997) Leptin accelerates the onset of puberty in normal female mice. J Clin Invest 99:391-5

Ahmed ML, Connors MH, Drayer NM, Jones JS, Dunger DB (1998) Pubertal growth in IDDM is determined by HbA1c levels, sex, and bone age. Diabetes Care 21:831-5

Ahmed ML, Ong KK, Morrell DJ, Cox L, Drayer N, Perry L, Preece MA, Dunger DB (1999) Longitudinal study of leptin concentrations during puberty: sex differences and relationship to changes in body composition. J Clin Endocrinol Metab 84:899-905

Ahmed ML, Ong KK, Watts AP, Morrell DJ, Preece MA, Dunger DB (2001) Elevated leptin levels are associated with excess gains in fat mass in girls, but not boys, with type 1 diabetes: longitudinal study during adolescence. J Clin Endocrinol Metab 86:1188-93

Ainslie DA, Morris MJ, Wittert G, Turnbull H, Proietto J, Thorburn AW (2001) Estrogen deficiency causes central leptin insensitivity and increased hypothalamic neuropeptide Y. Int J Obes Relat Metab Disord 25:1680-8

Albertsson-Wikland K, Rosberg S, Karlberg J, Groth T (1994) Analysis of 24-hour growth hormone profiles in healthy boys and girls of normal stature: relation to puberty. J Clin Endocrinol Metab 78:1195-201

Ambrosius WT, Compton JA, Bowsher RR, Pratt JH (1998) Relation of race, age, and sex hormone differences to serum leptin concentrations in children and adolescents. Horm Res 49:240-6

Amiel SA, Caprio S, Sherwin RS, Plewe G, Haymond MW, Tamborlane WV (1991) Insulin resistance of puberty: a defect restricted to peripheral glucose metabolism. J Clin Endocrinol Metab 72:277-82

Amiel SA, Sherwin RS, Hintz RL, Gertner JM, Press CM, Tamborlane WV (1984) Effect of diabetes and its control on insulin-like growth factors in the young subject with type I diabetes. Diabetes 33:1175-9

Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV (1986) Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. N Engl J Med 315:215-9

Anderson DC (1974) Sex-hormone-binding globulin. Clin Endocrinol (Oxf) 3:69-96

Anonymous (1988) Weight gain associated with intensive therapy in the diabetes control and complications trial. The DCCT Research Group. Diabetes Care 11:567-573

Anonymous (1989) Height at onset of IDDM in high and low risk countries. Japan and Pittsburgh Childhood Diabetes Research Groups.
Diab Res Clin Prac 6:173-176

Anonymous (1995) Adverse events and their association with treatment regimes in the diabetes control and complications trial. The DCCT Research Group. Diabetes Care 18:1415-1427

Apter D (1997) Leptin in puberty. Clin Endocrinol (Oxf) 47:175-6

Apter D, Bolton NJ, Hammond GL, Vihko R (1984) Serum sex hormone-binding globulin during puberty in girls and in different types of adolescent menstrual cycles. Acta Endocrinol (Copenh) 107:413-9

Argente J, Caballo N, Barrios V, Munoz MT, Pozo J, Chowen JA, Morande G, Hernandez M (1997) Multiple endocrine abnormalities of the growth hormone and insulin-like growth factor axis in patients with anorexia nervosa: effect of short- and long-term weight recuperation. J Clin Endocrinol Metab 82:2084-92

Arslanian S, Suprasongsin C (1996) Insulin sensitivity, lipids, and body composition in childhood: is "syndrome X" present? J Clin Endocrinol Metab 81:1058-62

Arslanian S, Suprasongsin C (1997) Testosterone treatment in adolescents with delayed puberty: changes in body composition, protein, fat, and glucose metabolism. J Clin Endocrinol Metab 82:3213-20

Attie KM, Ramirez NR, Conte FA, Kaplan SL, Grumbach MM (1990) The pubertal growth spurt in eight patients with true precocious puberty and growth hormone deficiency: evidence for a direct role of sex steroids. J Clin Endocrinol Metab 71:975-83

Balducci R, Finocchi G, Mangiantini A, Maggi C, Bianchi P, Guglielmi R, Toscano V (1992) Lack of correlation between sex hormone binding globulin, adrenal and peripheral androgens in precocious adrenarche. J Endocrinol Invest 15:501-5

Bartsch W, Horst HJ, Derwahl DM (1980) Interrelationships between sex hormone-binding globulin and 17 beta-estradiol, testosterone, 5 alpha-dihydrotestosterone, thyroxine, and triiodothyronine in prepubertal and pubertal girls. J Clin Endocrinol Metab 50:1053-6

Batch JA, Baxter RC, Werther G (1991) Abnormal regulation of insulin-like growth factor binding proteins in adolescents with insulin-dependent diabetes. J Clin Endocrinol Metab 73:964-8

Baxter-Jones AD, Mirwald RL, McKay HA, Bailey DA (2003) A longitudinal analysis of sex differences in bone mineral accrual in healthy 8-19-year-old boys and girls. Ann Hum Biol 30:160-75

Baxter RC, Bryson JM, Turtle JR (1980) Somatogenic receptors of rat liver: regulation by insulin. Endocrinology 107:1176-81

Baxter RC, Turtle JR (1978) Regulation of hepatic growth hormone receptors by insulin. Biochem Biophys Res Commun 84:350-7

Bereket A, Lang CH, Blethen SL, Ng LC, Wilson TA (1996) Insulin treatment normalizes reduced free insulin-like growth factor-I concentrations in diabetic children. Clin Endocrinol (Oxf) 45:321-6

Berkey CS, Reed RB, Valadian I (1983) Midgrowth spurt in height of Boston children. Ann Hum Biol 10:25-30

Bernasconi S, Vanelli M, Nori G, Siracusano MF, Marcellini C, Butturini A, De Luca F (1984) Serum TSH, T4, T3, FT4, FT3, rT3, and TBG in youngsters with non-ketotic insulin-dependent diabetes mellitus. Horm Res 20:213-7

Beunen G, Cameron N (1980) The reproducibility of TW2 skeletal age assessments by a self-taught assessor. Ann Hum Biol 7:155-62

Beunen G, Malina RM, Lefevre J, Claessens AL, Renson R, Simons J, Maes H, Vanreusel B, Lysens R (1994) Size, fatness and relative fat distribution of males of contrasting maturity status during adolescence and as adults. Int J Obes Relat Metab Disord 18:670-8

Bhasin S, Storer TW, Berman N, Yarasheski KE, Clevenger B, Phillips J, Lee WP, Bunnell TJ, Casaburi R (1997) Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. J Clin Endocrinol Metab 82:407-13

Biason-Lauber A, Zachmann M, Schoenle EJ (2000) Effect of leptin on CYP17 enzymatic activities in human adrenal cells: new insight in the onset of adrenarche. Endocrinology 141:1446-54

Bideci A, Cinaz P, Ezgu FS (2002) Leptin levels in children with insulin dependent diabetes mellitus. Turk J Pediatr 44:211-4

Bilezikian JP, Morishima A, Bell J, Grumbach MM (1998) Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med 339:599-603

Biro FM, Lucky AW, Simbartl LA, Barton BA, Daniels SR, Striegel-Moore R, Kronsberg SS, Morrison JA (2003) Pubertal maturation in girls and the relationship to anthropometric changes: pathways through puberty. J Pediatr 142:643-6

Biro FM, McMahon RP, Striegel-Moore R, Crawford PB, Obarzanek E, Morrison JA, Barton BA, Falkner F (2001) Impact of timing of pubertal maturation on growth in black and white female adolescents: The National Heart, Lung, and Blood Institute Growth and Health Study. J Pediatr 138:636-43

Bland JM, Altman DG (1995) Calculating correlation coefficients with repeated observations: Part 1--Correlation within subjects. BMJ 310:446

Blizzard RM, Martha PM, Kerrigan JR, Mauras N, Rogol AD (1989) Changes in growth hormone (GH) secretion and in growth during puberty. J Endocrinol Invest 12:65-8

Bloch CA, Clemons P, Sperling MA (1987) Puberty decreases insulin sensitivity. J Pediatr 110:481-7

Blogowska A, Rzepka-Gorska I, Krzyzanowska-Swiniarska B (2003) Growth hormone, IGF-1, insulin, SHBG, and estradiol levels in girls before menarche. Arch Gynecol Obstet 268:293-6

Blom L, Persson LA, Dahlquist G (1992) A high linear growth is associated with an increased risk of childhood diabetes mellitus. Diabetologia 35:528-33

Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Muller J, Skakkebaek NE, Heiman ML, Birkett M, Attanasio AM, Kiess W, Rascher W (1997) Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. J Clin Endocrinol Metab 82:2904-10

Bock RD (2004) Multiple prepubertal growth spurts in children of the Fels Longitudinal Study: comparison with results from the Edinburgh Growth Study. Ann Hum Biol 31:59-74

Bognetti E, Macellaro P, Novelli D, Meschi F, Ciralli F, Chiumello G (1995) Prevalence and correlates of obesity in insulin dependent diabetic patients. Arch Dis Child 73:239-42

Bognetti E, Riva MC, Bonfanti R, Meschi F, Viscardi M, Chiumello G (1998) Growth changes in children and adolescents with short-term diabetes. Diabetes Care 21:1226-9

Bolton NJ (1984) Sex Hormone Binding Globulin-A New Dimension in the Evaluation of Disorders of Androgen Action. Farmos Diagnostica Newletters

Bourguignon JP, Franchimont P (1984) Puberty-related increase in episodic LHRH release from rat hypothalamus in vitro. Endocrinology 114:1941-3

Bridges NA, Matthews DR, Hindmarsh PC, Brook CG (1994) Changes in gonadotrophin secretion during childhood and puberty. J Endocrinol 141:169-76
Brismar K, Fernqvist-Forbes E, Wahren J, Hall K (1994) Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. J Clin Endocrinol Metab 79:872-8
Brook CG (1971) Determination of body composition of children from skinfold measurements. Arch Dis Child 46:182-4
Brown M, Ahmed ML, Clayton KL, Dunger DB (1994) Growth during childhood and final height in type 1 diabetes. Diabet Med 11:182-7
Buckler J (1990) A Longitudinal Study of Adolescent Growth. Springer-Verlag, London, UK
Burkart W, Fischer-Guntenhoner E, Standl E, Schneider HP (1989) [Menarche, menstrual cycle and fertility in diabetic patients]. Geburtshilfe Frauenheilkd 49:149-54
Burns EC, Tanner JM, Preece MA, Cameron N (1981) Final height and pubertal development in 55 children with idiopathic growth hormone deficiency, treated for between 2 and 15 years with human growth hormone. Eur J Pediatr 137:155-64
Butler GE, McKie M, Ratcliffe SG (1990) The cyclical nature of prepubertal growth. Ann Hum Biol 17:177-98
Cameron N (1984) The measurement of human growth. Croom Helm, Beckenham, Kent, UK
Cameron N, Demerath EW (2002) Critical periods in human growth and their relationship to diseases of aging. Am J Phys Anthropol Suppl 35:159-84
Campfield LA, Smith FJ, Burn P (1996) The OB protein (leptin) pathway--a link between adipose tissue mass and central neural networks. Horm Metab Res 28:619-32
Caprio S (1999) Insulin: the other anabolic hormone of puberty. Acta Paediatr Suppl 88:84-7
Caprio S, Bronson M, Sherwin RS, Rife F, Tamborlane WV (1996) Co-existence of severe insulin resistance and hyperinsulinaemia in pre-adolescent obese children. Diabetologia 39:1489-97
Caprio S, Cline G, Boulware S, Permanente C, Shulman GI, Sherwin RS, Tamborlane WV (1994) Effects of puberty and diabetes on metabolism of insulin-sensitive fuels. Am J Physiol 266:E885-91
Caprio S, Plewe G, Diamond MP, Simonson DC, Boulware SD, Sherwin RS, Tamborlane WV (1989) Increased insulin secretion in puberty: a compensatory response to reductions in insulin sensitivity. J Pediatr 114:963-7
Cara JF, Rosenfield RL, Furlanetto RW (1987) A longitudinal study of the relationship of plasma somatomedin-C concentration to the pubertal growth spurt. Am J Dis Child 141:562-4
Carel JC, Lahlou N, Roger M, Chaussain JL (2004) Precocious puberty and statural growth. Hum Reprod Update 10:135-47
Carlsson B, Ankarberg C, Rosberg S, Norjavaara E, Albertsson-Wikland K, Carlsson LM (1997) Serum leptin concentrations in relation to pubertal development. Arch Dis Child 77:396-400
Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV (1996) Leptin: the tale of an obesity gene. Diabetes 45:1455-62

Carroll PV, Drake WM, Maher KT, Metcalfe K, Shaw NJ, Dunger DB, Cheetham TD, Camacho-Hubner C, Savage MO, Monson JP (2004) Comparison of continuation or cessation of growth hormone (GH) therapy on body composition and metabolic status in adolescents with severe GH deficiency at completion of linear growth. J Clin Endocrinol Metab 89:3890-5

Casabiell X, Pineiro V, Peino R, Lage M, Camina J, Gallego R, Vallejo LG, Dieguez C, Casanueva FF (1998) Gender differences in both spontaneous and stimulated leptin secretion by human omental adipose tissue in vitro: dexamethasone and estradiol stimulate leptin release in women, but not in men. J Clin Endocrinol Metab 83:2149-55

Chehab FF, Mounzih K, Lu R, Lim ME (1997) Early onset of reproductive function in normal female mice treated with leptin. Science 275:88-90

Chessler SD, Fujimoto WY, Shofer JB, Boyko EJ, Weigle DS (1998) Increased plasma leptin levels are associated with fat accumulation in Japanese Americans. Diabetes 47:239-43

Cheung CC, Thornton JE, Kuijper JL, Weigle DS, Clifton DK, Steiner RA (1997) Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinology 138:855-8

Chiarelli F, Tumini S, Verrotti A, Morgese G (1989) Effects of ketoacidosis and puberty on basal and TRH-stimulated thyroid hormones and TSH in children with diabetes mellitus. Horm Metab Res 21:494-7

Christensen L, Hagen C, Henriksen JE, Haug E (1997) Elevated levels of sex hormones and sex hormone binding globulin in male patients with insulin dependent diabetes mellitus. Effect of improved blood glucose regulation. Dan Med Bull 44:547-50

Chumlea WC, Guo SS (2002) The Assessment of Human Growth. In: Cameron N (ed) Human Growth And Development. Academic Press, London, pp 349-361

Cianfarani S, Bonfanti R, Bitti ML, Germani D, Boemi S, Chiumello G, Boscherini B (2000) Growth and insulin-like growth factors (IGFs) in children with insulin-dependent diabetes mellitus at the onset of disease: evidence for normal growth, age dependency of the IGF system alterations, and presence of a small (approximately 18-kilodalton) IGF-binding protein-3 fragment in serum. J Clin Endocrinol Metab 85:4162-7

Cicognani A, Zappulla F, Bernardi F, Capelli M, Mazzanti L, Turchi S, Radetti G, Pirazzoli P, Cacciari E (1978) Hypophyso-gonadal function in the diabetic child. Acta Paediatr Scand 67:151-5

Clarson C, Daneman D, Ehrlich RM (1985) The relationship of metabolic control to growth and pubertal development in children with insulin-dependent diabetes. Diabetes Res 2:237-41

Clayton KL, Holly JM, Carlsson LM, Jones J, Cheetham TD, Taylor AM, Dunger DB (1994) Loss of the normal relationships between growth hormone, growth hormone-binding protein and insulin-like growth factor-I in adolescents with insulin-dependent diabetes mellitus. Clin Endocrinol (Oxf) 41:517-24

Clayton PE, Gill MS, Hall CM, Tillmann V, Whatmore AJ, Price DA (1997) Serum leptin through childhood and adolescence. Clin Endocrinol (Oxf) 46:727-33

Clayton PE, Hall CM (2004) Insulin-like growth factor I levels in healthy children. Horm Res 62 Suppl 1:2-7

Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guy-Grand B (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392:398-401

Codner E, Barrera A, Mook-Kanamori D, Bazaes RA, Unanue N, Gaete X, Avila A, Ugarte F, Torrealba I, Perez V, Panteon E, Cassorla F (2004) Ponderal gain, waist-to-hip ratio, and pubertal development in girls with type-1 diabetes mellitus. Pediatr Diabetes 5:182-9

Codner E, Escobar-Morreale HF (2007) Clinical review: Hyperandrogenism and polycystic ovary syndrome in women with type 1 diabetes mellitus. J Clin Endocrinol Metab 92:1209-16

Codner E, Mook-Kanamori D, Bazaes RA, Unanue N, Sovino H, Ugarte F, Avila A, Iniguez G, Cassorla F (2005) Ovarian function during puberty in girls with type 1 diabetes mellitus: response to leuprolide. J Clin Endocrinol Metab 90:3939-45

Cohen HN, Paterson KR, Wallace AM, Beastall GH, Manderson WG, MacCuish AC (1984) Dissociation of adrenarche and gonadarche in diabetes mellitus. Clin Endocrinol (Oxf) 20:717-24

Cole TJ, Freeman JV, Preece MA (1995) Body mass index reference curves for the UK, 1990. Arch Dis Child 73:25-9

Cole TJ, Green PJ (1992) Smoothing reference centile curves: the LMS method and penalized likelihood. Stat Med 11:1305-19

Connors MH, Dunger DB, Chapel H, Jefferson I, Jowett TP, Edwards PR (1996) Diminished thyroxine-binding globulin in pubertal diabetic children. Diabetes Care 19:246-8

Conte FA, Grumbach MM, Kaplan SL, Reiter EO (1980) Correlation of luteinizing hormone-releasing factor-induced luteinizing hormone and follicle-stimulating hormone release from infancy to 19 years with the changing pattern of gonadotropin secretion in agonadal patients: relation to the restraint of puberty. J Clin Endocrinol Metab 50:163-8

Cook JS, Hoffman RP, Stene MA, Hansen JR (1993) Effects of maturational stage on insulin sensitivity during puberty. J Clin Endocrinol Metab 77:725-30

Corvol MT, Carrascosa A, Tsagris L, Blanchard O, Rappaport R (1987) Evidence for a direct in vitro action of sex steroids on rabbit cartilage cells during skeletal growth: influence of age and sex. Endocrinology 120:1422-9

Coste J, Ecosse E, Lesage C, Chaussain JL, Carel JC (2002) Evaluation of adolescent statural growth in health and disease: reliability of assessment from height measurement series and development of an automated algorithm. Horm Res 58:105-14

Couch RM (1992) Dissociation of cortisol and adrenal androgen secretion in poorly controlled insulin-dependent diabetes mellitus. Acta Endocrinol (Copenh) 127:115-7

Coutant R, de Casson FB, Rouleau S, Douay O, Mathieu E, Gatelais F, Bouhours-Nouet N, Voinot C, Audran M, Limal JM (2004) Divergent effect of endogenous and exogenous sex steroids on the insulin-like growth factor I response to growth hormone in short normal adolescents. J Clin Endocrinol Metab 89:6185-92

Dabadghao P, Vidmar S, Cameron FJ (2001) Deteriorating diabetic control through adolescence-do the origins lie in childhood? Diabet Med 18:889-94

Danielson KK, Palta M, Allen C, D'Alessio DJ (2005) The association of increased total glycosylated hemoglobin levels with delayed age at menarche in young women with type 1 diabetes. J Clin Endocrinol Metab 90:6466-71

Danne T, Kordonouri O, Enders I, Weber B (1997) Factors influencing height and weight development in children with diabetes. Results of the Berlin Retinopathy Study. Diabetes Care 20:281-5

Daughaday WH (1997) Sulfation factor revisited: the one-two punch of insulin-like growth factor-I action on cartilage. J Lab Clin Med 129:398-9

De Pergola G, Cospite MR, Giagulli VA, Giorgino F, Garruti G, Cignarelli M, Giorgino R (1993) Insulin-like growth factor-1 (IGF-1) and dehydroepiandrosterone sulphate in obese women. Int J Obes Relat Metab Disord 17:481-3

de Ridder CM, Thijssen JH, Bruning PF, Van den Brande JL, Zonderland ML, Erich WB (1992) Body fat mass, body fat distribution, and pubertal development: a longitudinal study of physical and hormonal sexual maturation of girls. J Clin Endocrinol Metab 75:442-6

Delemarre-van de Waal HA (2002) Regulation of puberty. Best Pract Res Clin Endocrinol Metab 16:1-12

Delemarre-van de Waal HA, van Coeverden SC, Rotteveel J (2001) Hormonal determinants of pubertal growth. J Pediatr Endocrinol Metab 14 Suppl 6:1521-6

Denburg MR, Silfen ME, Manibo AM, Chin D, Levine LS, Ferin M, McMahon DJ, Go C, Oberfield SE (2002) Insulin sensitivity and the insulin-like growth factor system in prepubertal boys with premature adrenarche. J Clin Endocrinol Metab 87:5604-9

Deurenberg P, Weststrate JA, Seidell JC (1991) Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. Br J Nutr 65:105-14

Dietz WH, Robinson TN (1998) Use of the body mass index (BMI) as a measure of overweight in children and adolescents. J Pediatr 132:191-3

Djursing H, Hagen C, Nyboe Andersen A, Svenstrup B, Bennett P, Molsted Pedersen L (1985) Serum sex hormone concentrations in insulin dependent diabetic women with and without amenorrhoea. Clin Endocrinol (Oxf) 23:147-54

Djursing H, Nyholm HC, Hagen C, Carstensen L, Pedersen LM (1982) Clinical and hormonal characteristics in women with anovulation and insulin-treated diabetes mellitus. Am J Obstet Gynecol 143:876-82

Domargard A, Sarnblad S, Kroon M, Karlsson I, Skeppner G, Aman J (1999) Increased prevalence of overweight in adolescent girls with type 1 diabetes mellitus. Acta Paediatr 88:1223-8

Donaghue KC, Kordonouri O, Chan A, Silink M (2003) Secular trends in growth in diabetes: are we winning? Arch Dis Child 88:151-4

Dorchy H, Bourdoux P, Lemiere B (1985) Subclinical thyroid hormone abnormalities in type I diabetic children and adolescents. Relationship to metabolic control. Acta Paediatr Scand 74:386-9

Drash AL (1993) The child, the adolescent, and the Diabetes Control and Complications Trial. Diabetes Care 16:1515-6

Drayer NM (1974) Height of diabetic children at onset of symptoms. Arch Dis Child 49:616-20
Du Caju MV, Rooman RP, op de Beeck L (1995) Longitudinal data on growth and final height in diabetic children. Pediatr Res 38:607-11
Dunger DB (1992) Diabetes in puberty. Arch Dis Child 67:569-70
Dunger DB, Cheetham TD (1996) Growth hormone insulin-like growth factor I axis in insulin-dependent diabetes mellitus. Horm Res 46:2-6
Dunger DB, Cheetham TD, Holly JM, Matthews DR (1993) Does recombinant insulin-like growth factor I have a role in the treatment of insulin-dependent diabetes mellitus during adolescence? Acta Paediatr Suppl 388:49-52; discussion 53
Dunger DB, Perkins JA, Jowett TP, Edwards PR, Cox LA, Preece MA, Ekins RP (1990) A longitudinal study of total and free thyroid hormones and thyroxine binding globulin during normal puberty. Acta Endocrinol (Copenh) 123:305-10
Durnin JV, Rahaman MM (1967) The assessment of the amount of fat in the human body from measurements of skinfold thickness. Br J Nutr 21:681-9
Eakman GD, Dallas JS, Ponder SW, Keenan BS (1996) The effects of testosterone and dihydrotestosterone on hypothalamic regulation of growth hormone secretion. J Clin Endocrinol Metab 81:1217-23
Ebeling P, Stenman UH, Seppala M, Koivisto VA (1995) Androgens and insulin resistance in type 1 diabetic men. Clin Endocrinol (Oxf) 43:601-7
Edelsten AD, Hughes IA, Oakes S, Gordon IR, Savage DC (1981) Height and skeletal maturity in children with newly-diagnosed juvenile-onset diabetes. Arch Dis Child 56:40-4
Edge JA, Dunger DB, Matthews DR, Gilbert JP, Smith CP (1990) Increased overnight growth hormone concentrations in diabetic compared with normal adolescents. J Clin Endocrinol Metab 71:1356-62
Elbers JM, Asscheman H, Seidell JC, Frolich M, Meinders AE, Gooren LJ (1997) Reversal of the sex difference in serum leptin levels upon cross-sex hormone administration in transsexuals. J Clin Endocrinol Metab 82:3267-70
Ellis KJ (2000) Human body composition: in vivo methods. Physiol Rev 80:649-80
Emmerson AJ, Savage DC (1988) Height at diagnosis in diabetes. Eur J Pediatr 147:319-20
Escobar-Morreale HF, Roldan B, Barrio R, Alonso M, Sancho J, de la Calle H, Garcia-Robles R (2000) High prevalence of the polycystic ovary syndrome and hirsutism in women with type 1 diabetes mellitus. J Clin Endocrinol Metab 85:4182-7
Evans N, Robinson VP, Lister J (1972) Growth and bone age of juvenile diabetics. Arch Dis Child 47:589-93
Fattah DI, Chard T (1981) Simplified method for measuring sex-hormone binding globulin. Clin Chem 27:1277-9
Field A (2005) Discovering Statistics Using SPSS. Sage Publications Ltd, London
Finkelstein JW, Roffwarg HP, Boyar RM, Kream J, Hellman L (1972) Age-related change in the twenty-four-hour spontaneous secretion of growth hormone. J Clin Endocrinol Metab 35:665-70

Forbes G (1978) Body composition in adolescence. In: Falkner F, Tanner JM (eds) Human Growth. Vol 2. Bailliere Tindall, London, pp 239-272
Forbes GB (1992) Body size and composition of perimenarchal girls. Am J Dis Child 146:63-6
Fowelin J, Attvall S, von Schenck H, Smith U, Lager I (1991) Characterization of the insulin-antagonistic effect of growth hormone in man. Diabetologia 34:500-6
Freeman JV, Cole TJ, Chinn S, Jones PR, White EM, Preece MA (1995) Cross sectional stature and weight reference curves for the UK, 1990. Arch Dis Child 73:17-24
Frisch RE, Revelle R (1970) Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science 169:397-9
Frisch RE, Revelle R (1971) Height and weight at menarche and a hypothesis of menarche. Arch Dis Child 46:695-701
Fung KP, Lee J, Lau SP, Chow OK, Wong TW, Davis DP (1990) Properties and clinical implications of body mass indices. Arch Dis Child 65:516-9
Galloway PJ, Donaldson MD, Wallace AM (2001) Sex hormone binding globulin concentration as a prepubertal marker for hyperinsulinaemia in obesity. Arch Dis Child 85:489-91
Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF (1997) Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. J Clin Endocrinol Metab 82:2849-55
Garrow JS, Webster J (1985) Quetelet's index (W/H2) as a measure of fatness. Int J Obes 9:147-53
Gasser T, Muller HG, Kohler W, Prader A, Largo R, Molinari L (1985) An analysis of the mid-growth and adolescent spurts of height based on acceleration. Ann Hum Biol 12:129-48
Gasser T, Sheehy A, Molinari L, Largo RH (2000) Sex dimorphism in growth. Ann Hum Biol 27:187-97
Gilani BB, MacGillivray MH, Voorhess ML, Mills BJ, Riley WJ, MacLaren NK (1984) Thyroid hormone abnormalities at diagnosis of insulin-dependent diabetes mellitus in children. J Pediatr 105:218-22
Gleeson H, Barreto ES, Salvatori R, Costa L, Oliveira CR, Pereira RM, Clayton P, Aguiar-Oliveira MH (2007) Metabolic effects of growth hormone (GH) replacement in children and adolescents with severe isolated GH deficiency due to a GHRH receptor mutation. Clin Endocrinol (Oxf) 66:466-74
Goldstein H (1995) The multilevel analysis of growth data. In: Hauspie R, Lindgren G, Falkner F (eds) Essays on Auxology. Castlemead Publications, Welwyn Garden City
Gower BA, Nagy TR, Goran MI (1999) Visceral fat, insulin sensitivity, and lipids in prepubertal children. Diabetes 48:1515-21
Grant DB (1967) Fasting serum insulin levels in childhood. Arch Dis Child 42:375-8
Gregory JW, Wilson AC, Greene SA (1992) Body fat and overweight among children and adolescents with diabetes mellitus. Diabet Med 9:344-8
Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, Macisaac RJ, Clarke S, Zajac JD, Jerums G (2008) Low testosterone levels are common and associated with insulin resistance in men with diabetes. J Clin Endocrinol Metab 93:1834-40

Grumbach MM, Styne DM (2003) Puberty: Ontogeny, Neuroendocrinology, Physiology, and Disorders. In: Larsen PR, Kronenberg, H.M, Melmed, S., Polonsky, K.S. (ed) Williams Textbook of Endocrinology. Saunders, Philadelphia, pp 1115-1286

Guercio G, Rivarola MA, Chaler E, Maceiras M, Belgorosky A (2002) Relationship between the GH/IGF-I axis, insulin sensitivity, and adrenal androgens in normal prepubertal and pubertal boys. J Clin Endocrinol Metab 87:1162-9

Guercio G, Rivarola MA, Chaler E, Maceiras M, Belgorosky A (2003) Relationship between the growth hormone/insulin-like growth factor-I axis, insulin sensitivity, and adrenal androgens in normal prepubertal and pubertal girls. J Clin Endocrinol Metab 88:1389-93

Gunczler P, Lanes R, Esaa S, Paoli M (1996) Effect of glycemic control on the growth velocity and several metabolic parameters of conventionally treated children with insulin dependent diabetes mellitus. J Pediatr Endocrinol Metab 9:569-75

Guo SM, Roche AF, Houtkooper L (1989) Fat-free mass in children and young adults predicted from bioelectric impedance and anthropometric variables. Am J Clin Nutr 50:435-43

Hagg U, Taranger J (1991) Height and height velocity in early, average and late maturers followed to the age of 25: a prospective longitudinal study of Swedish urban children from birth to adulthood. Ann Hum Biol 18:47-56

Halldin MU, Tylleskar K, Hagenas L, Tuvemo T, Gustafsson J (1998) Is growth hormone hypersecretion in diabetic adolescent girls also a daytime problem? Clin Endocrinol (Oxf) 48:785-94

Harris DA, Van Vliet G, Egli CA, Grumbach MM, Kaplan SL, Styne DM, Vainsel M (1985) Somatomedin-C in normal puberty and in true precocious puberty before and after treatment with a potent luteinizing hormone-releasing hormone agonist. J Clin Endocrinol Metab 61:152-9

Hauspie R (2002) Adolescence: Somatic Growth And Sex Differences. In: Cameron N (ed) Human Growth And Development. Academic Press, London, pp 45-63

Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller W, Phinney SD (1996) Gender differences in plasma leptin concentrations. Nat Med 2:949-50

Heinze HJ, Lowitt S, DeClue TJ, Malone JI (1993) Blunting of the pubertal growth spurt with an extended period of linear growth through age 20 in subjects with type 1 diabetes mellitus. Pediatr Res 33:1134

Herber SM, Dunsmore IR (1988) Does control affect growth in diabetes mellitus? Acta Paediatr Scand 77:303-5

Hernandez-Cassis C, Cure-Cure C, Lopez-Jaramillo P (1995) Effect of thyroid replacement therapy on the stature of Colombian children with minimal thyroid dysfunction. Eur J Clin Invest 25:454-6

Hibi I, Tanaka T, Tanae A, Kagawa J, Hashimoto N, Yoshizawa A, Shizume K (1989) The influence of gonadal function and the effect of gonadal suppression treatment on final height in growth hormone (GH)-treated GH-deficient children. J Clin Endocrinol Metab 69:221-6

Hindmarsh P, Di Silvio L, Pringle PJ, Kurtz AB, Brook CG (1988a) Changes in serum insulin concentration during puberty and their relationship to growth hormone. Clin Endocrinol (Oxf) 28:381-8

Hindmarsh PC (2002) Endocrinology Of Growth. In: Cameron N (ed) Human Growth And Development. Academic Press, London, pp 85-101

Hindmarsh PC, Brook CGD (1995) Normal Growth and its Endocrine Control. In: Brook CGD (ed) Clinical Paediatric Endocrinology. Blackwell Science Ltd, London, pp 85-106

Hindmarsh PC, Matthews DR, Brook CG (1988b) Growth hormone secretion in children determined by time series analysis. Clin Endocrinol (Oxf) 29:35-44

Hindmarsh PC, Matthews DR, Di Silvio L, Kurtz AB, Brook CG (1988c) Relation between height velocity and fasting insulin concentrations. Arch Dis Child 63:665-6

Hjelt K, Braendholt V, Kamper J, Vestermark S (1983) Growth in children with diabetes mellitus. The significance of metabolic control, insulin requirements and genetic factors. Dan Med Bull 30:28-33

Ho KY, Evans WS, Blizzard RM, Veldhuis JD, Merriam GR, Samojlik E, Furlanetto R, Rogol AD, Kaiser DL, Thorner MO (1987) Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. J Clin Endocrinol Metab 64:51-8

Hoffman RP, Vicini P, Sivitz WI, Cobelli C (2000) Pubertal adolescent male-female differences in insulin sensitivity and glucose effectiveness determined by the one compartment minimal model. Pediatr Res 48:384-8

Holl RW, Grabert M, Heinze E, Sorgo W, Debatin KM (1998a) Age at onset and long-term metabolic control affect height in type-1 diabetes mellitus. Eur J Pediatr 157:972-7

Holl RW, Grabert M, Sorgo W, Heinze E, Debatin KM (1998b) Contributions of age, gender and insulin administration to weight gain in subjects with IDDM. Diabetologia 41:542-7

Holl RW, Heinze E, Seifert M, Grabert M, Teller WM (1994) Longitudinal analysis of somatic development in paediatric patients with IDDM: genetic influences on height and weight. Diabetologia 37:925-9

Holl RW, Swift PG, Mortensen HB, Lynggaard H, Hougaard P, Aanstoot HJ, Chiarelli F, Daneman D, Danne T, Dorchy H, Garandeau P, Greene S, Hoey HM, Kaprio EA, Kocova M, Martul P, Matsuura N, Robertson KJ, Schoenle EJ, Sovik O, Tsou RM, Vanelli M, Aman J (2003) Insulin injection regimens and metabolic control in an international survey of adolescents with type 1 diabetes over 3 years: results from the Hvidore study group. Eur J Pediatr 162:22-9

Holly JM, Biddlecombe RA, Dunger DB, Edge JA, Amiel SA, Howell R, Chard T, Rees LH, Wass JA (1988) Circadian variation of GH-independent IGF-binding protein in diabetes mellitus and its relationship to insulin. A new role for insulin? Clin Endocrinol (Oxf) 29:667-75

Holly JM, Dunger DB, al-Othman SA, Savage MO, Wass JA (1992) Sex hormone binding globulin levels in adolescent subjects with diabetes mellitus. Diabet Med 9:371-4

Holly JM, Smith CP, Dunger DB, Howell RJ, Chard T, Perry LA, Savage MO, Cianfarani S, Rees LH, Wass JA (1989) Relationship between the pubertal fall in sex hormone binding globulin and insulin-like growth factor binding protein-I. A synchronized approach to pubertal development? Clin Endocrinol (Oxf) 31:277-84

Hoskins PJ, Leslie RD, Pyke DA (1985) Height at diagnosis of diabetes in children: a study in identical twins. Br Med J (Clin Res Ed) 290:278-80

Houtkooper LB (1996) Assessment of body composition in youths and relationship to sport. Int J Sport Nutr 6:146-64
Huang CY, Lee YJ, Huang FY, Hsu CH, Kao HA (2001) Final height of children with type 1 diabetes: the effects of age at diagnosis, metabolic control, and parental height. Acta Paediatr Taiwan 42:33-8
Hypponen E, Virtanen SM, Kenward MG, Knip M, Akerblom HK (2000) Obesity, increased linear growth, and risk of type 1 diabetes in children. Diabetes Care 23:1755-60
Ibanez L, Dimartino-Nardi J, Potau N, Saenger P (2000) Premature adrenarche--normal variant or forerunner of adult disease? Endocr Rev 21:671-96
Ibanez L, Potau N, Zampolli M, Rique S, Saenger P, Carrascosa A (1997) Hyperinsulinemia and decreased insulin-like growth factor-binding protein-1 are common features in prepubertal and pubertal girls with a history of premature pubarche. J Clin Endocrinol Metab 82:2283-8
Ibanez L, Virdis R, Potau N, Zampolli M, Ghizzoni L, Albisu MA, Carrascosa A, Bernasconi S, Vicens-Calvet E (1992) Natural history of premature pubarche: an auxological study. J Clin Endocrinol Metab 74:254-7
Illig R, Prader A (1970) Effect of testosterone on growth hormone secretion in patients with anorchia and delayed puberty. J Clin Endocrinol Metab 30:615-8
Ilondo MM, Vanderschueren-Lodeweyckx M, Vlietinck R, Pizarro M, Malvaux P, Eggermont E, Eeckels R (1982) Plasma androgens in children and adolescents. Part I: control subjects. Horm Res 16:61-77
Ingberg CM, Sarnblad S, Palmer M, Schvarcz E, Berne C, Aman J (2003) Body composition in adolescent girls with type 1 diabetes. Diabet Med 20:1005-11
Iuliano-Burns S, Mirwald RL, Bailey DA (2001) Timing and magnitude of peak height velocity and peak tissue velocities for early, average, and late maturing boys and girls. Am J Hum Biol 13:1-8
Jaaskelainen J, Voutilainen R (1997) Growth of patients with 21-hydroxylase deficiency: an analysis of the factors influencing adult height. Pediatr Res 41:30-3
Jivani SK, Rayner PH (1973) Does control influence the growth of diabetic children? Arch Dis Child 48:109-15
Jockenhovel F, Blum WF, Vogel E, Englaro P, Muller-Wieland D, Reinwein D, Rascher W, Krone W (1997) Testosterone substitution normalizes elevated serum leptin levels in hypogonadal men. J Clin Endocrinol Metab 82:2510-3
Johnson MS, Figueroa-Colon R, Huang TT, Dwyer JH, Goran MI (2001) Longitudinal changes in body fat in African American and Caucasian children: influence of fasting insulin and insulin sensitivity. J Clin Endocrinol Metab 86:3182-7
Johnston FE, Malina RM, Galbraith MA, Frisch RE, Revelle R, Cook S (1971) Height, weight and age at menarche and the "critical weight" hypothesis. Science 174:1148-9
Jos J, Meteyer I, Farkas D, Oberkampf B (1996) [Growth of children with insulin-dependent diabetes. Study of 104 cases]. Arch Pediatr 3:218-26
Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jorgensen K, Muller J, Hall K, Skakkebaek NE (1994) Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab 78:744-52

Juul A, Scheike T, Nielsen CT, Krabbe S, Muller J, Skakkebaek NE (1995) Serum insulin-like growth factor I (IGF-I) and IGF-binding protein 3 levels are increased in central precocious puberty: effects of two different treatment regimens with gonadotropin-releasing hormone agonists, without or in combination with an antiandrogen (cyproterone acetate). J Clin Endocrinol Metab 80:3059-67

Kalme T, Koistinen H, Loukovaara M, Koistinen R, Leinonen P, Angervo M, Seppala M (2003) Comparative studies on the regulation of insulin-like growth factor-binding protein-1 (IGFBP-1) and sex hormone-binding globulin (SHBG) production by insulin and insulin-like growth factors in human hepatoma cells. J Steroid Biochem Mol Biol 86:197-200

Kalme T, Loukovaara M, Koistinen R, Koistinen H, Angervo M, Leinonen P, Seppala M (1999) Estradiol increases the production of sex hormone-binding globulin but not insulin-like growth factor binding protein-1 in cultured human hepatoma cells. Fertil Steril 72:325-9

Kamoda T, Saitoh H, Nakahara S, Izumi I, Hirano T, Matsui A (1998) Serum leptin and insulin concentrations in prepubertal lean, obese and insulin-dependent diabetes mellitus children. Clin Endocrinol (Oxf) 49:385-9

Karaguzel G, Ozdem S, Boz A, Bircan I, Akcurin S (2006) Leptin levels and body composition in children and adolescents with type 1 diabetes. Clin Biochem 39:788-93

Karlberg J, Engstrom I, Karlberg P, Fryer JG (1987a) Analysis of linear growth using a mathematical model. I. From birth to three years. Acta Paediatr Scand 76:478-88

Karlberg J, Fryer JG, Engstrom I, Karlberg P (1987b) Analysis of linear growth using a mathematical model. II. From 3 to 21 years of age. Acta Paediatr Scand Suppl 337:12-29

Keenan BS, Richards GE, Ponder SW, Dallas JS, Nagamani M, Smith ER (1993) Androgen-stimulated pubertal growth: the effects of testosterone and dihydrotestosterone on growth hormone and insulin-like growth factor-I in the treatment of short stature and delayed puberty. J Clin Endocrinol Metab 76:996-1001

Kelnar CJ, Brook CG (1983) A mixed longitudinal study of adrenal steroid excretion in childhood and the mechanism of adrenarche. Clin Endocrinol (Oxf) 19:117-29

Kiess W, Anil M, Blum WF, Englaro P, Juul A, Attanasio A, Dotsch J, Rascher W (1998) Serum leptin levels in children and adolescents with insulin-dependent diabetes mellitus in relation to metabolic control and body mass index. Eur J Endocrinol 138:501-9

Kim MR, Gupta MK, Travers SH, Rogers DG, Van Lente F, Faiman C (1999) Serum prostate specific antigen, sex hormone binding globulin and free androgen index as markers of pubertal development in boys. Clin Endocrinol (Oxf) 50:203-10

Kirel B, Dogruel N, Korkmaz U, Kilic FS, Ozdamar K, Ucar B (2000) Serum leptin levels in type 1 diabetic and obese children: relation to insulin levels. Clin Biochem 33:475-80

Kjaer K, Hagen C, Sando SH, Eshoj O (1992) Epidemiology of menarche and menstrual disturbances in an unselected group of women with insulin-dependent diabetes mellitus compared to controls. J Clin Endocrinol Metab 75:524-9

Klein KO, Baron J, Colli MJ, McDonnell DP, Cutler GB, Jr. (1994) Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay. J Clin Invest 94:2475-80
Kobayashi T, Sawano S, Itoh T, Kosaka K, Hirayama H, Kasuya Y (1983) The pharmacokinetics of insulin after continuous subcutaneous infusion or bolus subcutaneous injection in diabetic patients. Diabetes 32:331-6
Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mudaliar SR, Olefsky J, Caro JF (1996) Acute and chronic effects of insulin on leptin production in humans: Studies in vivo and in vitro. Diabetes 45:699-701
Largo RH, Gasser T, Prader A, Stuetzle W, Huber PJ (1978) Analysis of the adolescent growth spurt using smoothing spline functions. Ann Hum Biol 5:421-34
Lautala P, Akerblom HK, Viikari J, Louhivuori K, Uhari M, Dahlstrom S, Dahl M, Lahde PL, Pesonen E, Pietikainen M, et al. (1985) Atherosclerosis precursors in Finnish children and adolescents. VII. Serum immunoreactive insulin. Acta Paediatr Scand Suppl 318:127-33
Le Roith D (1997) Seminars in medicine of the Beth Israel Deaconess Medical Center. Insulin-like growth factors. N Engl J Med 336:633-40
Lebl J, Schober E, Zidek T, Baldis S, Rami B, Pruhova S, Kolouskova S, Snajderova M, Frisch H (2003) Growth data in large series of 587 children and adolescents with type 1 diabetes mellitus. Endocr Regul 37:153-61
Lee T, Stewart-Brown S, Wadsworth J, Savage D (1984) Growth in children with diabetes. In: Borms J (ed) Human Growth and Development. Plenum Press, New York, pp 613-618
Legro RS, Lin HM, Demers LM, Lloyd T (2000) Rapid maturation of the reproductive axis during perimenarche independent of body composition. J Clin Endocrinol Metab 85:1021-5
LeRoith D, Sampson PC, Roberts CT, Jr. (1994) How does the mitogenic insulin-like growth factor I receptor differ from the metabolic insulin receptor? Horm Res 41 Suppl 2:74-8; discussion 79
Leslie RD, Lo S, Millward BA, Honour J, Pyke DA (1991) Decreased growth velocity before IDDM onset. Diabetes 40:211-6
Link K, Blizzard RM, Evans WS, Kaiser DL, Parker MW, Rogol AD (1986) The effect of androgens on the pulsatile release and the twenty-four-hour mean concentration of growth hormone in peripubertal males. J Clin Endocrinol Metab 62:159-64
Loche S, Cappa M, Borrelli P, Faedda A, Crino A, Cella SG, Corda R, Muller EE, Pintor C (1987) Reduced growth hormone response to growth hormone-releasing hormone in children with simple obesity: evidence for somatomedin-C mediated inhibition. Clin Endocrinol (Oxf) 27:145-53
Lofqvist C, Andersson E, Gelander L, Rosberg S, Blum WF, Albertsson Wikland K (2001) Reference values for IGF-I throughout childhood and adolescence: a model that accounts simultaneously for the effect of gender, age, and puberty. J Clin Endocrinol Metab 86:5870-6
Lohman TG (1986) Applicability of body composition techniques and constants for children and youths. Exerc Sport Sci Rev 14:325-57
Loukovaara M, Carson M, Adlercreutz H (1995) Regulation of production and secretion of sex hormone-binding globulin in HepG2 cell cultures by hormones and growth factors. J Clin Endocrinol Metab 80:160-4

Loviselli A, Pisanu P, Cossu E, Caradonna A, Massa GM, Cirillo R, Balestrieri A (1994) [Low levels of dehydroepiandrosterone sulfate in adult males with insulin-dependent diabetes mellitus]. Minerva Endocrinol 19:113-9
Luna AM, Wilson DM, Wibbelsman CJ, Brown RC, Nagashima RJ, Hintz RL, Rosenfeld RG (1983) Somatomedins in adolescence: a cross-sectional study of the effect of puberty on plasma insulin-like growth factor I and II levels. J Clin Endocrinol Metab 57:268-71
Luna R, Fluiters E, Rodriguez I, Garcia-Mayor RV (2004) Growth in Type 1 diabetic children. Diabet Med 21:1054-6
Luna R, Garcia-Mayor RV, Lage M, Andrade MA, Barreiro J, Pombo M, Dieguez C, Casanueva FF (1999) High serum leptin levels in children with type 1 diabetes mellitus: contribution of age, BMI, pubertal development and metabolic status. Clin Endocrinol (Oxf) 51:603-10
Maes M, Underwood LE, Ketelslegers JM (1986) Low serum somatomedin-C in insulin-dependent diabetes: evidence for a postreceptor mechanism. Endocrinology 118:377-82
Maffei M, Fei H, Lee GH, Dani C, Leroy P, Zhang Y, Proenca R, Negrel R, Ailhaud G, Friedman JM (1995) Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus. Proc Natl Acad Sci U S A 92:6957-60
Maffeis C, Moghetti P, Grezzani A, Clementi M, Gaudino R, Tato L (2002) Insulin resistance and the persistence of obesity from childhood into adulthood. J Clin Endocrinol Metab 87:71-6
Malhotra A, Poon E, Tse WY, Pringle PJ, Hindmarsh PC, Brook CG (1993) The effects of oxandrolone on the growth hormone and gonadal axes in boys with constitutional delay of growth and puberty. Clin Endocrinol (Oxf) 38:393-8
Mannucci E, Ognibene A, Becorpi A, Cremasco F, Pellegrini S, Ottanelli S, Rizzello SM, Massi G, Messeri G, Rotella CM (1998) Relationship between leptin and oestrogens in healthy women. Eur J Endocrinol 139:198-201
Mantzoros CS, Flier JS, Rogol AD (1997) A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. J Clin Endocrinol Metab 82:1066-70
Marshall WA, Tanner JM (1969) Variations in pattern of pubertal changes in girls. Arch Dis Child 44:291-303
Marshall WA, Tanner JM (1970) Variations in the pattern of pubertal changes in boys. Arch Dis Child 45:13-23
Martha PM, Jr., Rogol AD, Carlsson LM, Gesundheit N, Blizzard RM (1993) A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. I. Serum growth hormone-binding protein. J Clin Endocrinol Metab 77:452-7
Martha PM, Jr., Rogol AD, Veldhuis JD, Kerrigan JR, Goodman DW, Blizzard RM (1989) Alterations in the pulsatile properties of circulating growth hormone concentrations during puberty in boys. J Clin Endocrinol Metab 69:563-70
Martin LG, Grossman MS, Connor TB, Levitsky LL, Clark JW, Camitta FD (1979) Effect of androgen on growth hormone secretion and growth in boys with short stature. Acta Endocrinol (Copenh) 91:201-12

Maruyama Y, Aoki N, Suzuki Y, Ohno Y, Imamura M, Saika T, Sinohara H, Yamamoto T (1987) Sex-steroid-binding plasma protein (SBP), testosterone, oestradiol and dehydroepiandrosterone (DHEA) in prepuberty and puberty. Acta Endocrinol (Copenh) 114:60-7

Massa G, Dooms L, Bouillon R, Vanderschueren-Lodeweyckx M (1993) Serum levels of growth hormone-binding protein and insulin-like growth factor I in children and adolescents with type 1 (insulin-dependent) diabetes mellitus. Diabetologia 36:239-43

Mast M, Kortzinger I, Konig E, Muller MJ (1998) Gender differences in fat mass of 5-7-year old children. Int J Obes Relat Metab Disord 22:878-84

Mast M, Sonnichsen A, Langnase K, Labitzke K, Bruse U, Preub U, Muller MJ (2002) Inconsistencies in bioelectrical impedance and anthropometric measurements of fat mass in a field study of prepubertal children. Br J Nutr 87:163-75

Mauras N, Blizzard RM, Link K, Johnson ML, Rogol AD, Veldhuis JD (1987) Augmentation of growth hormone secretion during puberty: evidence for a pulse amplitude-modulated phenomenon. J Clin Endocrinol Metab 64:596-601

Mauras N, Rini A, Welch S, Sager B, Murphy SP (2003) Synergistic effects of testosterone and growth hormone on protein metabolism and body composition in prepubertal boys. Metabolism 52:964-9

Mauras N, Rogol AD, Haymond MW, Veldhuis JD (1996) Sex steroids, growth hormone, insulin-like growth factor-1: neuroendocrine and metabolic regulation in puberty. Horm Res 45:74-80

Maynard LM, Wisemandle W, Roche AF, Chumlea WC, Guo SS, Siervogel RM (2001) Childhood body composition in relation to body mass index. Pediatrics 107:344-50

McCarthy HD, Cole TJ, Fry T, Jebb SA, Prentice AM (2006) Body fat reference curves for children. Int J Obes (Lond) 30:598-602

Meredith HV, Spurgeon JH, Meredith EM (1981) Early seriatim research on human somatic growth. Growth 45:151-67

Messinis IE, Milingos S, Zikopoulos K, Kollios G, Seferiadis K, Lolis D (1998) Leptin concentrations in the follicular phase of spontaneous cycles and cycles superovulated with follicle stimulating hormone. Hum Reprod 13:1152-6

Meyer K, Deutscher J, Anil M, Berthold A, Bartsch M, Kiess W (2000) Serum androgen levels in adolescents with type 1 diabetes: relationship to pubertal stage and metabolic control. J Endocrinol Invest 23:362-8

Miell JP, Taylor AM, Zini M, Maheshwari HG, Ross RJ, Valcavi R (1993) Effects of hypothyroidism and hyperthyroidism on insulin-like growth factors (IGFs) and growth hormone- and IGF-binding proteins. J Clin Endocrinol Metab 76:950-5

Miller JD, Tannenbaum GS, Colle E, Guyda HJ (1982) Daytime pulsatile growth hormone secretion during childhood and adolescence. J Clin Endocrinol Metab 55:989-94

Miller JD, Wright NM, Lester SE, Felsing NE, Linzer J, Chan E, White DA, Charles MA (1992) Spontaneous and stimulated growth hormone release in adolescents with type I diabetes mellitus: effects of metabolic control. J Clin Endocrinol Metab 75:1087-91

Mitamura R, Yano K, Suzuki N, Ito Y, Makita Y, Okuno A (1999) Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, and testosterone

secretion before the onset of male puberty. J Clin Endocrinol Metab 84:29-37
Molinari L, Largo RH, Prader A (1980) Analysis of the growth spurt at age seven (mid-growth spurt). Helv Paediatr Acta 35:325-34
Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387:903-8
Montalto J, Yong AB, Funder JW, Connelly JF (1989) Serum 5-androstene-3 beta,17 beta-diol sulphate, sex hormone binding globulin and free androgen index in girls with premature adrenarche. J Steroid Biochem 33:1149-54
Morales A, Wasserfall C, Brusko T, Carter C, Schatz D, Silverstein J, Ellis T, Atkinson M (2004) Adiponectin and leptin concentrations may aid in discriminating disease forms in children and adolescents with type 1 and type 2 diabetes. Diabetes Care 27:2010-4
Moran A, Jacobs DR, Jr., Steinberger J, Cohen P, Hong CP, Prineas R, Sinaiko AR (2002) Association between the insulin resistance of puberty and the insulin-like growth factor-I/growth hormone axis. J Clin Endocrinol Metab 87:4817-20
Moran A, Jacobs DR, Jr., Steinberger J, Hong CP, Prineas R, Luepker R, Sinaiko AR (1999) Insulin resistance during puberty: results from clamp studies in 357 children. Diabetes 48:2039-44
Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K (1995) Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 80:3689-98
Morrell DJ, Dadi H, More J, Taylor AM, Dabestani A, Buchanan CR, Holder AT, Preece MA (1989) A monoclonal antibody to human insulin-like growth factor-I: characterization, use in radioimmunoassay and effect on the biological activities of the growth factor. J Mol Endocrinol 2:201-6
Mortensen HB, Hartling SG, Petersen KE (1988) A nation-wide cross-sectional study of glycosylated haemoglobin in Danish children with type 1 diabetes. Diabet Med 5:871-6
Mortensen HB, Hougaard P (1997) Comparison of metabolic control in a cross-sectional study of 2,873 children and adolescents with IDDM from 18 countries. The Hvidore Study Group on Childhood Diabetes. Diabetes Care 20:714-20
Myers SE, Albert SG, Haas MJ, Clifton D, Mooradian AD (2004) Pubertal changes in serum leptin levels in adolescents with type 1 diabetes mellitus: a controlled longitudinal study. J Pediatr Endocrinol Metab 17:1653-62
Nanto-Salonen K, Muller HL, Hoffman AR, Vu TH, Rosenfeld RG (1993) Mechanisms of thyroid hormone action on the insulin-like growth factor system: all thyroid hormone effects are not growth hormone mediated. Endocrinology 132:781-8
Neely EK, Beukers MW, Oh Y, Cohen P, Rosenfeld RG (1991) Insulin-like growth factor receptors. Acta Paediatr Scand Suppl 372:116-23; discussion 124

Neely EK, Hintz RL, Parker B, Bachrach LK, Cohen P, Olney R, Wilson DM (1992) Two-year results of treatment with depot leuprolide acetate for central precocious puberty. J Pediatr 121:634-40
Nestler JE, McClanahan MA, Clore JN, Blackard WG (1992) Insulin inhibits adrenal 17,20-lyase activity in man. J Clin Endocrinol Metab 74:362-7
Nestler JE, Usiskin KS, Barlascini CO, Welty DF, Clore JN, Blackard WG (1989) Suppression of serum dehydroepiandrosterone sulfate levels by insulin: an evaluation of possible mechanisms. J Clin Endocrinol Metab 69:1040-6
Ong K, Kratzsch J, Kiess W, Dunger D (2002) Circulating IGF-I levels in childhood are related to both current body composition and early postnatal growth rate. J Clin Endocrinol Metab 87:1041-4
Pal BR, Phillips PE, Matthews DR, Dunger DB (1992) Contrasting metabolic effects of continuous and pulsatile growth hormone administration in young adults with type 1 (insulin-dependent) diabetes mellitus. Diabetologia 35:542-9
Palmert MR, Hayden DL, Mansfield MJ, Crigler JF, Jr., Crowley WF, Jr., Chandler DW, Boepple PA (2001) The longitudinal study of adrenal maturation during gonadal suppression: evidence that adrenarche is a gradual process. J Clin Endocrinol Metab 86:4536-42
Palmert MR, Radovick S, Boepple PA (1998) The impact of reversible gonadal sex steroid suppression on serum leptin concentrations in children with central precocious puberty. J Clin Endocrinol Metab 83:1091-6
Parker LN (1991) Adrenarche. Endocrinol Metab Clin North Am 20:71-83
Pasquali R, Casimirri F, De Iasio R, Mesini P, Boschi S, Chierici R, Flamia R, Biscotti M, Vicennati V (1995) Insulin regulates testosterone and sex hormone-binding globulin concentrations in adult normal weight and obese men. J Clin Endocrinol Metab 80:654-8
Pelleymounter MA, Baker MB, McCaleb M (1999) Does estradiol mediate leptin's effects on adiposity and body weight? Am J Physiol 276:E955-63
Penfold J, Chase HP, Marshall G, Walravens CF, Walravens PA, Garg SK (1995) Final adult height and its relationship to blood glucose control and microvascular complications in IDDM. Diabet Med 12:129-33
Pescovitz OH, Rosenfeld RG, Hintz RL, Barnes K, Hench K, Comite F, Loriaux DL, Cutler GB, Jr. (1985) Somatomedin-C in accelerated growth of children with precocious puberty. J Pediatr 107:20-5
Petersen H, Korsgaard B, Deckert T, Nielsen E (1978) Growth, body weight and insulin requirements in diabetic children. Acta Paediatrica Scandinavica 67:453-7
Pietrobelli A, Faith MS, Allison DB, Gallagher D, Chiumello G, Heymsfield SB (1998) Body mass index as a measure of adiposity among children and adolescents: a validation study. J Pediatr 132:204-10
Pitukcheewanont P, Alemzadeh R, Jacobs WR, Jones BH, Eberle AJ (1995) Does glycemic control affect growth velocity in children with insulin-dependent diabetes mellitus. Acta Diabetol 32:148-52
Plymate SR, Matej LA, Jones RE, Friedl KE (1988) Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. J Clin Endocrinol Metab 67:460-4
Preece MA, Baines MJ (1978) A new family of mathematical models describing the human growth curve. Ann Hum Biol 5:1-24

Preece MA, Cameron N, Donmall M, Dunger D, Holder A, Preece J, Seth J, Sharp G, Taylor A (1984) The Endocrinology of Male Puberty. In: Borms J, Hauspie R, Sand A, Susanne C, Hebbellnok M (eds) Human Growth And Development. Plenum Publishing corp, pp 23-37
Prentice AM, Jebb SA (2001) Beyond body mass index. Obes Rev 2:141-7
Press M, Tamborlane WV, Sherwin RS (1984) Importance of raised growth hormone levels in mediating the metabolic derangements of diabetes. N Engl J Med 310:810-5
Price DE, Burden AC (1992) Growth of children before onset of diabetes. Diabetes Care 15:1393-5
Qureshi A, Kopelman PG (1997) Leptin--fat messenger or fat controller? Clin Endocrinol (Oxf) 47:169-71
Radetti G, Paganini C, Antoniazzi F, Pasquino B, Valentini R, Gentili L, Tato L (1997) Growth hormone-binding proteins, IGF-I and IGF-binding proteins in children and adolescents with type 1 diabetes mellitus. Horm Res 47:110-5
Radetti G, Paganini C, Gentili L, Barbin F, Pasquino B, Zachmann M (1994) Altered adrenal and thyroid function in children with insulin-dependent diabetes mellitus. Acta Diabetol 31:138-40
Ramachandran A, Snehalatha C, Joseph TA, Vijay V, Viswanathan M (1994) Height at onset of insulin-dependent diabetes in children in southern India. Diabetes Res Clin Pract 23:55-7
Ravussin E, Pratley RE, Maffei M, Wang H, Friedman JM, Bennett PH, Bogardus C (1997) Relatively low plasma leptin concentrations precede weight gain in Pima Indians. Nat Med 3:238-40
Reichard P, Berglund B, Britz A, Cars I, Nilsson BY, Rosenqvist U (1991) Intensified conventional insulin treatment retards the microvascular complications of insulin-dependent diabetes mellitus (IDDM): the Stockholm Diabetes Intervention Study (SDIS) after 5 years. J Intern Med 230:101-8
Reiter EO, Fuldauer VG, Root AW (1977) Secretion of the adrenal androgen, dehydroepiandrosterone sulfate, during normal infancy, childhood, and adolescence, in sick infants, and in children with endocrinologic abnormalities. J Pediatr 90:766-70
Reiter EO, Grumbach MM (1982) Neuroendocrine control mechanisms and the onset of puberty. Annu Rev Physiol 44:595-613
Remer T, Manz F (1999) Role of nutritional status in the regulation of adrenarche. J Clin Endocrinol Metab 84:3936-44
Remer T, Manz F (2001) The midgrowth spurt in healthy children is not caused by adrenarche. J Clin Endocrinol Metab 86:4183-6
Reynolds E, Wines J (1948) Individualized differences in physical changes associated with adolescence in girls. Amer J Dis Child 75:329-50
Reynolds EL, Wines JV (1951) Physical changes associated with adolescence in boys. AMA Am J Dis Child 82:529-47
Riad-Gabriel MG, Jinagouda SD, Sharma A, Boyadjian R, Saad MF (1998) Changes in plasma leptin during the menstrual cycle. Eur J Endocrinol 139:528-31
Rico H, Revilla M, Villa LF, Hernandez ER, Alvarez de Buergo M, Villa M (1993) Body composition in children and Tanner's stages: a study with dual-energy x-ray absorptiometry. Metabolism 42:967-70

Rizza RA, Mandarino LJ, Gerich JE (1982) Effects of growth hormone on insulin action in man. Mechanisms of insulin resistance, impaired suppression of glucose production, and impaired stimulation of glucose utilization. Diabetes 31:663-9

Roemmich JN, Clark PA, Weltman A, Veldhuis JD, Rogol AD (2005) Pubertal alterations in growth and body composition: IX. Altered spontaneous secretion and metabolic clearance of growth hormone in overweight youth. Metabolism 54:1374-83

Rogers DG, Sherman LD, Gabbay KH (1991) Effect of puberty on insulinlike growth factor I and HbA1 in type I diabetes. Diabetes Care 14:1031-5

Rohner-Jeanrenaud F, Cusin I, Sainsbury A, Zakrzewska KE, Jeanrenaud B (1996) The loop system between neuropeptide Y and leptin in normal and obese rodents. Horm Metab Res 28:642-8

Rolland-Cachera MF (1993) Body composition during adolescence: methods, limitations and determinants. Horm Res 39 Suppl 3:25-40

Rooman RP, De Beeck LO, Martin M, van Doorn J, Mohan S, Du Caju MV (2005) Ethinylestradiol and testosterone have divergent effects on circulating IGF system components in adolescents with constitutional tall stature. Eur J Endocrinol 152:597-604

Rose SR, Municchi G, Barnes KM, Kamp GA, Uriarte MM, Ross JL, Cassorla F, Cutler GB, Jr. (1991) Spontaneous growth hormone secretion increases during puberty in normal girls and boys. J Clin Endocrinol Metab 73:428-35

Rosenbaum M, Leibel RL (1999) Clinical review 107: Role of gonadal steroids in the sexual dimorphisms in body composition and circulating concentrations of leptin. J Clin Endocrinol Metab 84:1784-9

Rosner W (1990) The functions of corticosteroid-binding globulin and sex hormone-binding globulin: recent advances. Endocr Rev 11:80-91

Round JM, Jones DA, Honour JW, Nevill AM (1999) Hormonal factors in the development of differences in strength between boys and girls during adolescence: a longitudinal study. Ann Hum Biol 26:49-62

Rubin KR, Lichtenfels JM, Ratzan SK, Ozonoff M, Rowe DW, Carey DE (1986) Relationship of somatomedin-C concentration to bone age in boys with constitutional delay of growth. Am J Dis Child 140:555-8

Rudberg S, Persson B (1995) Indications of low sex hormone binding globulin (SHBG) in young females with type 1 diabetes, and an independent association to microalbuminuria. Diabet Med 12:816-22

Rudberg S, Persson B (1998) Serum leptin levels in young females with insulin-dependent diabetes and the relationship to hyperandrogenicity and microalbuminuria. Horm Res 50:297-302

Rudolf MC, Sherwin RS, Markowitz R, Bates SE, Genel M, Hochstadt J, Tamborlane WV (1982) Effect of intensive insulin treatment on linear growth in the young diabetic patient. J Pediatr 101:333-9

Saad RJ, Keenan BS, Danadian K, Lewy VD, Arslanian SA (2001) Dihydrotestosterone treatment in adolescents with delayed puberty: does it explain insulin resistance of puberty? J Clin Endocrinol Metab 86:4881-6

Salardi S, Fava A, Cassio A, Cicognani A, Tassoni P, Pirazzoli P, Frejaville E, Balsamo A, Cozzuti E, Cacciari E (1984) Thyroid function and prolactin levels in insulin-dependent diabetic children and adolescents. Diabetes 33:522-6

Salardi S, Tonioli S, Tassoni P, Tellarini M, Mazzanti L, Cacciari E (1987) Growth and growth factors in diabetes mellitus. Arch Dis Child 62:57-62
Salardi S, Zucchini S, Cicognani A, Gualandi S, Barbieri E, Cacciari E (2002) Inhibin B levels in adolescents and young adults with type 1 diabetes. Horm Res 57:205-8
Salerno M, Argenziano A, Di Maio S, Gasparini N, Formicola S, De Filippo G, Tenore A (1997) Pubertal growth, sexual maturation, and final height in children with IDDM. Effects of age at onset and metabolic control. Diabetes Care 20:721-4
Salmon WD, Jr., Burkhalter VJ (1997) Stimulation of sulfate and thymidine incorporation into hypophysectomized rat cartilage by growth hormone and insulin-like growth factor-I in vitro: the somatomedin hypothesis revisited. J Lab Clin Med 129:430-8
Salmon WD, Jr., Daughaday WH (1957) A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J Lab Clin Med 49:825-36
Savage MO, Smith CP, Dunger DB, Gale EA, Holly JM, Preece MA (1992) Insulin and growth factors adaptation to normal puberty. Horm Res 37 Suppl 3:70-3
Scheffer-Marinus PD, Links TP, Reitsma WD, Drayer NM (1999) Increased height in diabetes mellitus corresponds to the predicted and the adult height. Acta Paediatr 88:384-8
Schriock EA, Winter RJ, Traisman HS (1984) Diabetes mellitus and its effects on menarche. J Adolesc Health Care 5:101-4
Shimizu H, Shimomura Y, Hayashi R, Ohtani K, Sato N, Futawatari T, Mori M (1997a) Serum leptin concentration is associated with total body fat mass, but not abdominal fat distribution. Int J Obes Relat Metab Disord 21:536-41
Shimizu H, Shimomura Y, Nakanishi Y, Futawatari T, Ohtani K, Sato N, Mori M (1997b) Estrogen increases in vivo leptin production in rats and human subjects. J Endocrinol 154:285-92
Shishko PI, Kovalev PA, Goncharov VG, Zajarny IU (1992) Comparison of peripheral and portal (via the umbilical vein) routes of insulin infusion in IDDM patients. Diabetes 41:1042-9
Siervogel RM, Demerath EW, Schubert C, Remsberg KE, Chumlea WC, Sun S, Czerwinski SA, Towne B (2003) Puberty and body composition. Horm Res 60:36-45
Silfen ME, Manibo AM, Ferin M, McMahon DJ, Levine LS, Oberfield SE (2002) Elevated free IGF-I levels in prepubertal Hispanic girls with premature adrenarche: relationship with hyperandrogenism and insulin sensitivity. J Clin Endocrinol Metab 87:398-403
Singh A, Hamilton-Fairley D, Koistinen R, Seppala M, James VH, Franks S, Reed MJ (1990) Effect of insulin-like growth factor-type I (IGF-I) and insulin on the secretion of sex hormone binding globulin and IGF-I binding protein (IBP-I) by human hepatoma cells. J Endocrinol 124:R1-3
Siri WE (1956) The gross composition of the body. Adv Biol Med Phys 4:239-80
Sizonenko PC, Paunier L (1986) Failure of dehydroepiandrosterone enanthate to promote growth. J Clin Endocrinol Metab 62:1322-4
Sizonenko PC, Paunier L, Carmignac D (1976) Hormonal changes during puberty. IV. Longitudinal study of adrenal androgen secretions. Horm Res 7:288-302

Sklar CA, Kaplan SL, Grumbach MM (1980) Evidence for dissociation between adrenarche and gonadarche: studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotropin deficiency, and constitutionally delayed growth and adolescence. J Clin Endocrinol Metab 51:548-56

Sluszkiewicz E (1986) Thyroid function in insulin-dependent diabetic children. Exp Clin Endocrinol 87:345-8

Small M, Gray CE, Beastall GH, MacCuish AC (1989) Adrenal androgens in insulin-dependent diabetes mellitus. Diabetes Res 11:93-5

Smals AG, Kloppenborg PW, Benraad TJ (1974) Body proportions and androgenicity in relation to plasma testosterone levels in Klinefelter's syndrome. Acta Endocrinol (Copenh) 77:387-400

Smith CP, Archibald HR, Thomas JM, Tarn AC, Williams AJ, Gale EA, Savage MO (1988) Basal and stimulated insulin levels rise with advancing puberty. Clin Endocrinol (Oxf) 28:7-14

Smith CP, Dunger DB, Williams AJ, Taylor AM, Perry LA, Gale EA, Preece MA, Savage MO (1989) Relationship between insulin, insulin-like growth factor I, and dehydroepiandrosterone sulfate concentrations during childhood, puberty, and adult life. J Clin Endocrinol Metab 68:932-7

Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS (1994) Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 331:1056-61

Soliman AT, Omar M, Assem HM, Nasr IS, Rizk MM, El Matary W, El Alaily RK (2002) Serum leptin concentrations in children with type 1 diabetes mellitus: relationship to body mass index, insulin dose, and glycemic control. Metabolism 51:292-6

Songer TJ, LaPorte RE, Tajima N, Orchard TJ, Rabin BS, Eberhardt MS, Dorman JS, Cruickshanks KJ, Cavender DE, Becker DJ, et al. (1986) Height at diagnosis of insulin dependent diabetes in patients and their non-diabetic family members. Br Med J (Clin Res Ed) 292:1419-22

Sorensen K, Andersson AM, Skakkebaek NE, Juul A (2007) Serum sex hormone-binding globulin levels in healthy children and girls with precocious puberty before and during gonadotropin-releasing hormone agonist treatment. J Clin Endocrinol Metab 92:3189-96

Stanhope R, Brook CG (1988) An evaluation of hormonal changes at puberty in man. J Endocrinol 116:301-5

Stanhope R, Preece MA, Grant DB, Brook CG (1988) New concepts of the growth spurt of puberty. Acta Paediatr Scand Suppl 347:30-7

Stark O, Peckham CS, Moynihan C (1989) Weight and age at menarche. Arch Dis Child 64:383-7

Steiner DF, Cunningham D, Spigelman L, Aten B (1967) Insulin biosynthesis: evidence for a precursor. Science 157:697-700

Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, et al. (1995) The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 377:530-2

Strain G, Zumoff B, Rosner W, Pi-Sunyer X (1994) The relationship between serum levels of insulin and sex hormone-binding globulin in men: the effect of weight loss. J Clin Endocrinol Metab 79:1173-6

Strasser-Vogel B, Blum WF, Past R, Kessler U, Hoeflich A, Meiler B, Kiess W (1995) Insulin-like growth factor (IGF)-I and -II and IGF-binding proteins-

1, -2, and -3 in children and adolescents with diabetes mellitus: correlation with metabolic control and height attainment. J Clin Endocrinol Metab 80:1207-13
Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD (1998) A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet 18:213-5
Stuetzle W, Gasser T, Molinari L, Largo RH, Prader A, Huber PJ (1980) Shape-invariant modelling of human growth. Ann Hum Biol 7:507-28
Tam CS, de Zegher F, Garnett SP, Baur LA, Cowell CT (2006) Opposing influences of prenatal and postnatal growth on the timing of menarche. J Clin Endocrinol Metab 91:4369-73
Tanner J (1962) Growth at Adolescence. Blackwell Scientific Publications, Oxford
Tanner J (1989) Foetus into Man. Castlemead Publications, Ware, Herts
Tanner JM (1999) A short walk in the garden of auxological delights. In: Johnston FE ZB, Eveleth PB (ed) Human Growth in Context. Smith-Gordon Co Ltd, London, pp 1-14
Tanner JM, Buckler JM (1997) Revision and update of Tanner-Whitehouse clinical longitudinal charts for height and weight. Eur J Pediatr 156:248-9
Tanner JM, Cameron N (1980) Investigation of the mid-growth spurt in height, weight and limb circumferences in single-year velocity data from the London, 1966-67 growth survey. Ann Hum Biol 7:565-77
Tanner JM, Davies PS (1985) Clinical longitudinal standards for height and height velocity for North American children. J Pediatr 107:317-29
Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy M, Goldstein H (1983) Assessment of Skeletal Maturity and Prediction of Adult Height (TW2 Method). Academic Press, London, UK
Tanner JM, Whitehouse RH, Marubini E, Resele LF (1976) The adolescent growth spurt of boys and girls of the Harpenden growth study. Ann Hum Biol 3:109-26
Tanner JM, Whitehouse RH, Takaishi M (1966a) Standards from birth to maturity for height, weight, height velocity, and weight velocity: British children, 1965. I. Arch Dis Child 41:454-71
Tanner JM, Whitehouse RH, Takaishi M (1966b) Standards from birth to maturity for height, weight, height velocity, and weight velocity: British children, 1965. II. Arch Dis Child 41:613-35
Tattersall RB, Pyke DA (1973) Growth in diabetic children. Studies in identical twins. Lancet 2:1105-9
Taylor AM, Dunger DB, Grant DB, Preece MA (1988) Somatomedin-C/IGF-I measured by radioimmunoassay and somatomedin bioactivity in adolescents with insulin dependent diabetes compared with puberty matched controls. Diabetes Res 9:177-81
Taylor AM, Dunger DB, Preece MA, Holly JM, Smith CP, Wass JA, Patel S, Tate VE (1990) The growth hormone independent insulin-like growth factor-I binding protein BP-28 is associated with serum insulin-like growth factor-I inhibitory bioactivity in adolescent insulin-dependent diabetics. Clin Endocrinol (Oxf) 32:229-39
Taylor RW, Gold E, Manning P, Goulding A (1997) Gender differences in body fat content are present well before puberty. Int J Obes Relat Metab Disord 21:1082-4

Terasawa E, Fernandez DL (2001) Neurobiological mechanisms of the onset of puberty in primates. Endocr Rev 22:111-51
Thon A, Heinze E, Feilen KD, Holl RW, Schmidt H, Koletzko S, Wendel U, Nothjunge J (1992) Development of height and weight in children with diabetes mellitus: report on two prospective multicentre studies, one cross-sectional, one longitudinal. Eur J Pediatr 151:258-62
Tillmann V, Adojaan B, Shor R, Price DA, Tuvemo T (1996) Physical development in Estonian children with type 1 diabetes. Diabet Med 13:97-101
Tomar R, Dhindsa S, Chaudhuri A, Mohanty P, Garg R, Dandona P (2006) Contrasting testosterone concentrations in type 1 and type 2 diabetes. Diabetes Care 29:1120-2
Travers SH, Jeffers BW, Bloch CA, Hill JO, Eckel RH (1995) Gender and Tanner stage differences in body composition and insulin sensitivity in early pubertal children. J Clin Endocrinol Metab 80:172-8
Travers SH, Jeffers BW, Eckel RH (2002) Insulin resistance during puberty and future fat accumulation. J Clin Endocrinol Metab 87:3814-8
Treuth MS, Butte NF, Wong WW, Ellis KJ (2001) Body composition in prepubertal girls: comparison of six methods. Int J Obes Relat Metab Disord 25:1352-9
Trimarchi F, De Luca F, Vanelli M, Benvenga S, Siracusano MF, Volta C, Bernasconi S (1984) Circulating thyroid antibodies and thyroid function studies in children and adolescents with insulin-dependent diabetes mellitus. Eur J Pediatr 142:253-6
Tuominen JA, Ebeling P, Stenman UH, Heiman ML, Stephens TW, Koivisto VA (1997) Leptin synthesis is resistant to acute effects of insulin in insulin-dependent diabetes mellitus patients. J Clin Endocrinol Metab 82:381-2
Ulijaszek SJ, Kerr DA (1999) Anthropometric measurement error and the assessment of nutritional status. Br J Nutr 82:165-77
Uriarte MM, Baron J, Garcia HB, Barnes KM, Loriaux DL, Cutler GB, Jr. (1992) The effect of pubertal delay on adult height in men with isolated hypogonadotropic hypogonadism. J Clin Endocrinol Metab 74:436-40
Vaisse C, Halaas JL, Horvath CM, Darnell JE, Jr., Stoffel M, Friedman JM (1996) Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. Nat Genet 14:95-7
van den Broeck J (2001) Reliability of Measurements, Growth Analyser. Dutch Growth Foundation, Rotterdam
van Lenthe FJ, Kemper CG, van Mechelen W (1996a) Rapid maturation in adolescence results in greater obesity in adulthood: the Amsterdam Growth and Health Study. Am J Clin Nutr 64:18-24
van Lenthe FJ, Kemper HC, van Mechelen W, Post GB, Twisk JW, Welten DC, Snel J (1996b) Biological maturation and the distribution of subcutaneous fat from adolescence into adulthood: the Amsterdam Growth and Health Study. Int J Obes Relat Metab Disord 20:121-9
van Wieringen JC, Wafelbakker F, Vebrugge HP, de Haas JH (1968) Groediagrammen nederland 1965. Wolterns-Noordhoff, Groningen
Veldhuis JD, Frystyk J, Iranmanesh A, Orskov H (2005) Testosterone and estradiol regulate free insulin-like growth factor I (IGF-I), IGF binding protein 1 (IGFBP-1), and dimeric IGF-I/IGFBP-1 concentrations. J Clin Endocrinol Metab 90:2941-7

Veldhuis JD, Metzger DL, Martha PM, Jr., Mauras N, Kerrigan JR, Keenan B, Rogol AD, Pincus SM (1997) Estrogen and testosterone, but not a nonaromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. J Clin Endocrinol Metab 82:3414-20

Veldhuis JD, Roemmich JN, Richmond EJ, Bowers CY (2006) Somatotropic and gonadotropic axes linkages in infancy, childhood, and the puberty-adult transition. Endocr Rev 27:101-40

Veldhuis JD, Roemmich JN, Rogol AD (2000) Gender and sexual maturation-dependent contrasts in the neuroregulation of growth hormone secretion in prepubertal and late adolescent males and females--a general clinical research center-based study. J Clin Endocrinol Metab 85:2385-94

Verrotti A, Basciani F, Morgese G, Chiarelli F (1998) Leptin levels in non-obese and obese children and young adults with type 1 diabetes mellitus. Eur J Endocrinol 139:49-53

Vizmanos B, Marti-Henneberg C, Cliville R, Moreno A, Fernandez-Ballart J (2001) Age of pubertal onset affects the intensity and duration of pubertal growth peak but not final height. Am J Human Biol 13:409-16

von Schoultz B, Carlstrom K (1989) On the regulation of sex-hormone-binding globulin--a challenge of an old dogma and outlines of an alternative mechanism. J Steroid Biochem 32:327-34

Wabitsch M, Blum WF, Muche R, Braun M, Hube F, Rascher W, Heinze E, Teller W, Hauner H (1997) Contribution of androgens to the gender difference in leptin production in obese children and adolescents. J Clin Invest 100:808-13

Wang Y (2002) Is obesity associated with early sexual maturation? A comparison of the association in American boys versus girls. Pediatrics 110:903-10

Wathen NC, Perry LA, Rubenstein E, Chard T (1987) A relationship between sex hormone binding globulin and dehydroepiandrosterone sulfate in normally menstruating females. Gynecol Endocrinol 1:47-50

Wattigney WA, Srinivasan SR, Chen W, Greenlund KJ, Berenson GS (1999) Secular trend of earlier onset of menarche with increasing obesity in black and white girls: the Bogalusa Heart Study. Ethn Dis 9:181-9

Wells JC (2000) A Hattori chart analysis of body mass index in infants and children. Int J Obes Relat Metab Disord 24:325-9

Wells JC (2001) A critique of the expression of paediatric body composition data. Arch Dis Child 85:67-72

Wells JC (2007) Sexual dimorphism of body composition. Best Pract Res Clin Endocrinol Metab 21:415-30

Weststrate JA, Deurenberg P (1989) Body composition in children: proposal for a method for calculating body fat percentage from total body density or skinfold-thickness measurements. Am J Clin Nutr 50:1104-15

WHO MGRSG (2006) Reliability of anthropometric measurements in the WHO Multicentre Growth Reference Study. Acta Paediatr Suppl 450:38-46

Wickman S, Saukkonen T, Dunkel L (2002) The role of sex steroids in the regulation of insulin sensitivity and serum lipid concentrations during male puberty: a prospective study with a P450-aromatase inhibitor. Eur J Endocrinol 146:339-46

Wildt L, Marshall G, Knobil E (1980) Experimental induction of puberty in the infantile female rhesus monkey. Science 207:1373-5

Wilson DM, Killen JD, Hammer LD, Litt IF, Vosti C, Miner B, Hayward C, Taylor CB (1991) Insulin-like growth factor-I as a reflection of body composition, nutrition, and puberty in sixth and seventh grade girls. J Clin Endocrinol Metab 73:907-12

Wise JE, Kolb EL, Sauder SE (1992) Effect of glycemic control on growth velocity in children with IDDM. Diabetes Care 15:826-30

Yki-Jarvinen H, Makimattila S, Utriainen T, Rutanen EM (1995) Portal insulin concentrations rather than insulin sensitivity regulate serum sex hormone-binding globulin and insulin-like growth factor binding protein 1 in vivo. J Clin Endocrinol Metab 80:3227-32

Yosha S, Fay M, Longcope C, Braverman LE (1984) Effect of D-thyroxine on serum sex hormone binding globulin (SHBG), testosterone, and pituitary-thyroid function in euthyroid subjects. J Endocrinol Invest 7:489-94

Zacharias L, Rand WM (1983) Adolescent growth in height and its relation to menarche in contemporary American girls. Ann Hum Biol 10:209-22

Zachmann M, Prader A, Sobel EH, Crigler JF, Jr., Ritzen EM, Atares M, Ferrandez A (1986) Pubertal growth in patients with androgen insensitivity: indirect evidence for the importance of estrogens in pubertal growth of girls. J Pediatr 108:694-7

Zachrisson I, Brismar K, Hall K, Wallensteen M, Dahlqvist G (1997) Determinants of growth in diabetic pubertal subjects. Diabetes Care 20:1261-5

Zemel BS (2002) Body Composition During Growth And Development. In: Cameron N (ed) Human Growth And Development. Academic Press, London, pp 271-293

Zemel BS, Katz SH (1986) The contribution of adrenal and gonadal androgens to the growth in height of adolescent males. Am J Phys Anthropol 71:459-66

Zhang LH, Rodriguez H, Ohno S, Miller WL (1995) Serine phosphorylation of human P450c17 increases 17,20-lyase activity: implications for adrenarche and the polycystic ovary syndrome. Proc Natl Acad Sci U S A 92:10619-23

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-32

Zumoff B, Miller L, Poretsky L, Levit CD, Miller EH, Heinz U, Denman H, Jandorek R, Rosenfeld RS (1990) Subnormal follicular-phase serum progesterone levels and elevated follicular-phase serum estradiol levels in young women with insulin-dependent diabetes. Steroids 55:560-4